**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
**WASHINGTON, D.C. 20460**

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**TXR No. 0050321**

MEMORANDUM

DATE: 12/14/2004

SUBJECT: 044309. Clothianidin Toxicology Data Evaluation Records

PC Code: 044309 DP Barcode: D282578

TO: Dan Kenny
RM 01
Registration Division (7505C)

FROM: Pamela M. Hurley, Toxicologist
Reregistration Branch 3
Health Effects Division (7509C)

THRU: Richard Loranger, Senior Scientist
Registration Action Branch 2
Health Effects Division (7509C)

Background and Summary:

The Health Effects Division (HED) was asked to review all the submitted toxicology studies conducted with the new chemical, Clothianidin (TI-435). All the Data Evaluation Records (DERs) reviewed by HED are attached. The acute toxicity studies on the technical material have been previously reviewed by the Registration Division (RD); however, some of the acute toxicity studies conducted with metabolites/degradates were reviewed by HED. They are included in this package.

With the exception of a developmental immunotoxicity study, the toxicological database for Clothianidin is adequate for assessment of risk to infants and children. There is a concern for immunotoxicity following exposure of clothianidin during the period of organogenesis. This concern was based on the decreases in absolute and adjusted thymus and spleen weights observed in several species in various studies. In addition, the available data indicate that the juvenile rats appeared to be more sensitive/susceptible to these effects than adults in the two-generation

reproduction study. Testing needs to be conducted to assess immune system function in adults and young animals following exposure during the period of organogenesis.

The requirements (CFR 158.340) for food use for Clothianidin are listed in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used. Table 1 lists studies conducted with the technical material and Table 2 lists additional studies conducted with metabolites and degradates.

**Table 1. Clothianidin Guideline Requirements: Studies Conducted with Technical Material**

| Test | | Technical | | |
|---|---|---|---|---|
| | | MRID | Required | Satisfied |
| 870.1100 | Acute Oral Toxicity | 45422621 | yes | yes |
| 870.1200 | Acute Dermal Toxicity | 45422634 | yes | yes |
| 870.1300 | Acute Inhalation Toxicity | 45422636 | yes | yes |
| 870.2400 | Primary Eye Irritation | 45422701 | yes | yes |
| 870.2500 | Primary Dermal Irritation | 45422703 | yes | yes |
| 870.2600 | Dermal Sensitization | 45422705 | yes | yes |
| 870.3100 | Oral Subchronic (rodent) | 45422708, 45422809 | yes | yes |
| 870.3150 | Oral Subchronic (nonrodent) | 45422808, -10, -11 | yes | yes |
| 870.3200 | 21-Day Dermal | 45422707 | yes | yes |
| 870.3250 | 90-Day Dermal | Not submitted | no | - |
| 870.3465 | 90-Day Inhalation | Not submitted | no | - |
| 870.3700a | Developmental Toxicity (rodent) | 45422710 and -11 | yes | yes |
| 870.3700b | Developmental Toxicity (nonrodent) | 45422712 and -13 | yes | yes |
| 870.3800 | Reproduction | 45422714 thru -16, 45422825 thru -26 | yes | yes |
| 870.4100a | Chronic Toxicity (rodent) | 45422719 and -20 | yes | yes[a] |
| 870.4100b | Chronic Toxicity (nonrodent) | 45422717 and -18 | yes | yes |
| 870.4200a | Oncogenicity (rat) | 45422719 and - 20 | yes | yes |
| 870.4200b | Oncogenicity (mouse) | 45422709, 45422721, 45422722 | yes | yes |
| 870.4300 | Chronic/Oncogenicity | 45422719, 45422720 | yes | yes |
| 870.5100 | Mutagenicity—Gene Mutation - bacterial | 45422731<br>45422732<br>45422733<br>45422734 | yes | yes |
| 870.5300 | Mutagenicity—Gene Mutation - mammalian | 45422736<br>45422737<br>45422738 | yes | yes |
| 870.5375 | Mutagenicity—Structural Chromosomal Aberrations | 45422740 | yes | yes |
| 870.5550 | Mutagenicity—Other Genotoxic Effects | 45422735<br>45422739 | yes | yes |

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

| Table and Group | Strain | | | | |
|---|---|---|---|---|---|
| | TA 1535 | TA 100 | TA 1537 | TA 98 | TA 102 |
| 1-5 µg/Plate | | | | | |
| 0 | 10 | 84 | 9 | 18 | 250 |
| 16 | 10 | 81 | 6 | 19 | 259 |
| 50 | 7 | 80 | 6 | 16 | 263 |
| 158 | 7 | 81 | 5 | 13 | 294 |
| 500 | 7 | 82 | 6 | 20 | 257 |
| 1581 | 10 | 73 | 6 | 12 | 256 |
| 5000 | 10 | 63 | 4 | 18 | 196 |
| Na-azide | 729 | | | | |
| NF | | 248 | | | |
| 4-NPDA | | | 97 | 171 | |
| Cumene | | | | | 400 |
| 6-10 µg/Tube | | | | | |
| 0 | 7 | 66 | 9 | 21 | 241 |
| 16 | 8 | 64 | 9 | 23 | 278 |
| 50 | 8 | 66 | 10 | 17 | 257 |
| 158 | 7 | 76 | 10 | 19 | 255 |
| 500 | 6 | 78 | 10 | 24 | 251 |
| 1581 | 8 | 55 | 9 | 17 | 235 |
| 5000 | - | 41 | 4 | 13 | 132 |
| Na-azide | 572 | | | | |
| NF | | 220 | | | |
| 4-NPDA | | | 169 | 172 | |
| Cumene | | | | | 511 |



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| Test | Technical | | |
|---|---|---|---|
| | MRID | Required | Satisfied |
| 870.6100a Acute Delayed Neurotox. (hen) | Not submitted | no | - |
| 870.6100b 90-Day Neurotoxicity (hen) | Not submitted | no | - |
| 870.6200a Acute Neurotox. Screening Battery (rat) | 45422801 and -02 | yes | yes |
| 870.6200b 90 Day Neuro. Screening Battery (rat) | 45422803, 45422825 | yes | yes |
| 870.6300 Developmental Neurotoxicity | 45422804 | yes | yes |
| Developmental Immunotoxicity | Not yet submitted | yes | no |
| 870.7485 General Metabolism | 45422805 and -06<br>45422807<br>45422823 | yes | yes |
| 870.7600 Dermal Penetration | 45868001 | no | yes |
| Special Studies for Ocular Effects<br>Acute Oral (rat)<br>Subchronic Oral (rat)<br>Six-month Oral (dog) | Not submitted | no | - |

a Satisfied with combined chronic toxicity/carcinogenicity study

# DATA EVALUATION RECORD

TI 435 (CLOTHIANIDIN)

Study Type (§83-6a): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 4-02-191A (MRID 45422804)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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**EPA Reviewer:** Susan Makris **Signature:** Susan Makris
**Toxicology Branch, Health Effects Division (7509C)** **Date** 11-21-04
**EPA Work Assignment Manager:** Ghazi Dannan, Ph.D. **Signature:** Ghazi Dannan
**Registration Action Branch 3, Health Effects Division (7509C)** **Date** 1/6/05

**TXR#:** 0050321

# DATA EVALUATION RECORD

**STUDY TYPE:** Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

**PC CODE:** 044309 **DP BARCODE:** D282308
**SUBMISSION NO.:** S613887

**TEST MATERIAL (PURITY):** TI 435 (Clothianidin, 95.5-95.9% a.i.)

**SYNONYMS:** (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

**CITATION:** Hoberman, A.M. (2000) Developmental neurotoxicity study of TI 435 administered orally via the diet to CRL:CD® presumed pregnant rats. Argus Research Laboratories, Inc., Horsham, PA. Laboratory Project ID: 1120-003, October 20, 2000. MRID 45422804. Unpublished.

**SPONSOR:** Takeda Chemical Industries Ltd. Agro Company, 13-10, Nihonbashi 2-chome, Chuo-Ku, Toyko 103, Japan

**EXECUTIVE SUMMARY:** In a developmental neurotoxicity study (MRID 45422804) TI 435 (clothianidin, 95.5-95.9% a.i.; Batch # LOT30037120-97) was administered in the diet to pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats (25/dose) from gestation day (GD) 0 to lactation day (LD) 22 at doses of 0, 150, 500, or 1750 ppm (0, 12.9, 42.9, and 142 mg/kg/day during gestation; 0, 27.3, 90.0, and 299.0 mg/kg/day during lactation). The day that litter delivery was completed was designated postnatal day (PND) 1 (or LD 1). Body weight, and food consumption data were recorded for dams. Although the dams were not subjected to a formal functional observational battery (FOB), detailed clinical observations included most of the FOB parameters. Dams were killed and necropsied on LD 22. On PND 5, litters were standardized to yield 5 males and 5 females (as closely as possible), and 10 randomly selected pups/sex/group were subjected to detailed clinical examination outside the home cage. On PND 12, pups were randomly assigned to each of the following four subsets: 1) fixed brain weights and/or neuropathological evaluation on PND 12 (10/sex/group); 2) passive avoidance testing (on PND 24-25 and 31-32) and water maze testing (on PND 59-61 and 66-68) (20/sex/group); 3) motor activity testing (on PND 14, 18, 22, and 60) and auditory startle habituation (on PND 23 and 61) (20/sex/group); 4) detailed clinical exam outside the home cage on PND 12 and weekly during PND 22-79 (20/sex/group), fixed brain weights and neuropathological evaluation on PND 80-83

(10/sex/group). In addition, the pups from subsets 2-4 were observed for the age of attainment of balanopreputial separation or vaginal patency (60/sex/group).

At 1750 ppm, maternal body weights were consistently decreased ($p \leq 0.05$ or NS) throughout gestation and lactation (↓2-8%). Body weight gains were decreased ($p \leq 0.05$) during GDs 0-3 (↓63%) and LDs 4-7 (↓67%). These decreases corresponded with the significant reductions noted in absolute (↓7-23%) and relative (↓6-22%) food consumption during the gestation and lactation periods. These findings were considered to be minimal in nature. There were no treatment-related effects on maternal survival, clinical or functional observations, or reproductive function at 1750 ppm. No treatment-related findings were observed in the 500 or 150 ppm groups.

**The maternal LOAEL is 1750 ppm (142 mg/kg/day) based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 500 ppm (42.9 mg/kg/day).**

No treatment-related differences in live litter size, postnatal survival (through PND 22), sex ratios, clinical signs, food consumption, sexual maturation, or physical landmarks were observed in any treated group. Learning, memory, brain weights, gross pathology, neuropathology, and morphometric measurements were unaffected by the test substance. It was stated that no treatment-related FOB findings were observed; however, no FOB data were provided.

Significantly decreased body weight and body weight gains were observed from approximately PND 14-22 in female pups at 500 ppm and in both sexes at 1750 ppm. Overall pre-weaning (PND 5-22) body weight gains were decreased ($p \leq 0.05$) by 7% in the 500 ppm females and by 18% at 1750 ppm in both sexes, as compared to controls. Immediately after weaning, decreased ($p \leq 0.05$) body weights were noted at 1750 ppm in both sexes (↓4-15%), and two male and three female weanlings died (PNDs 25-27). However, within 3 weeks post-weaning (i.e., following the cessation of treatment) the treatment-related body weight deficits in 1750 ppm pups had essentially been reversed.

There were no effects of treatment on other developmental landmarks examined, including pinna unfolding, eye opening, pupil constriction, acoustic startle response, surface righting reflex, balanopreputial separation or vaginal patency. No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance or water maze tests.

On PND 22, a treatment-related decrease (↓24%; not statistically significant) in mean total motor activity (number of movements) and mean time spent in movement was observed in the 1750 ppm males. There did not appear to be any treatment-related differences in habituation between control and treated rats. There were no apparent effects of treatment in motor activity data measured on PND 14, 18, or 62.

In the 1750 ppm females on PND 23, the average magnitude of the acoustic startle response over 5 blocks and the mean response for each individual block were substantially decreased ($p \leq 0.01$)

by 45-50% (average of 48%) as compared to controls. This finding was considered to be treatment-related.

There were no treatment-related differences in brain weights or brain-to-body weight ratios between control and treated groups for offspring at PND 12 or at termination (PND 83-87). No treatment-related macroscopic findings were noted at necropsy and no treatment-related microscopic findings were noted at subjective histopathological evaluation of nervous system tissues. Morphometric evaluations of PND 12 pup brains revealed an increased thickness of the hippocampal gyrus (4% in males and 9% in females), an increased thickness of the cerebellum height (4% in males and 10% in females), and a decreased thickness of the external germinal layer of the cerebellum (11%) at PND 12. In adult offspring at termination (PND 83-87), the thickness of the hippocampal gyrus was decreased for both males and females (5%) and the measurement of the caudate putamen was decreased 6% in females.

**The offspring systemic LOAEL is 500 ppm (42.9 mg/kg/day), based on decreased body weights and body weight gains of female pups during PND 14-21. The offspring systemic NOAEL is 150 ppm (12.9 mg/kg/day).**

**The offspring neurotoxicity LOAEL is 1750 ppm (142 mg/kg/day), based on decreased motor activity (number and duration of movements) in PND 22 male pups, decreased magnitude of the auditory startle response in PND 23 females, increases in the thickness of the hippocampal gyrus and cerebellum height and decreases in the external germinal layer in the brains of PND 12 pups; and decreases in the thickness of the caudate putamen and hippocampal gyrus in adult offspring at termination (PND 83-87). The offspring neurotoxicity NOAEL is 500 ppm (42.9 mg/kg/day).**

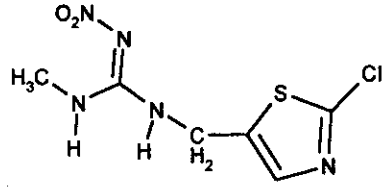
This study is classified as **acceptable/non-guideline** and does not satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats. Classification may be upgradable to guideline upon submission of procedural information for functional observation assessments.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS

**1. Test Material:** TI 435 technical (Clothianidin)

| | |
|---|---|
| **Description:** | Pale yellow powder |
| **Batch #:** | LOT30037120-97 |
| **Purity:** | 95.5-95.9 % a.i. |
| **Compound Stability:** | It was stated that the test substance was stable in the diet for at least 7 days at room temperature or refrigerated for 28 days. |
| **CAS # of TGAI:** | 210880-92-5 |
| **Structure:** |  |

**2. Vehicle:** Diet

**3. Test animals (P)**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | Crl:CD®(SD)IGS BR VAF/Plus® | |
| **Age at study initiation:** | Approximately 10 weeks | |
| **Wt. at study initiation:** | 207-254 g (females) | |
| **Source:** | Charles River Laboratories, Inc., Portage, MI | |
| **Housing:** | Following successful mating, females were housed individually in nesting boxes with Bed-o'cobs® bedding. The females and offspring were housed together in these cages through lactation day (LD) 21. After weaning, offspring selected for evaluation of developmental landmarks, neurobehavioral testing, and neuropathological examination were housed individually in wire bottomed cages until their scheduled termination. | |
| **Diet:** | Purina Mills Rodent LabChow® 5001-4 in "etts" form (PMI Nutrition International Inc., St. Louis, MO), *ad libitum* | |
| **Water:** | Reverse osmosis, chlorine-treated tap water, *ad libitum* | |
| **Environmental conditions:** | **Temperature:** | 18-26 °C (nominal) |
| | **Humidity:** | 30-70% (nominal) |
| | **Air changes:** | Approximately 10/hour |
| | **Photoperiod:** | Not reported |
| **Acclimation period:** | 6 days | |

## B. PROCEDURES AND STUDY DESIGN

**1. In life dates** - Start: 4/20/99 End: 8/06/99

**2. Study schedule:** P females were administered the test substance continuously in the diet from gestation day (GD) 0 until LD 22. Females were allowed to deliver naturally; the day of completion of delivery was designated lactation day (postnatal day 1). All P females (including those that did not deliver) were killed on LD 22. On postnatal day (PND) 5, ten pups/litter were randomly selected in order to reduce variability among the litters; the remaining offspring were weighed and euthanized. On PND 12, twenty litters/dose were randomly selected for continued

examination, and the litters were standardized to 8 pups/litter. Subsequently, ten pups/sex/group were selected for neurobehavioral testing (Subsets 2 and 3) and neuropathological examination (Subsets 1 and 4). Pups not selected for behavioral evaluations (Subset 5) were sacrificed on PND 22. All pups in Subsets 2, 3, and 4 were evaluated for either vaginal patency or balanopreputial separation.

3. **Mating procedure:** At approximately 10 weeks of age, females were paired with males (1:1) for mating for up to 4 days. Successful mating was determined by the presence of a copulatory plug or sperm in a vaginal smear. The day on which successful mating was determined was designated as GD 0. Following successful mating, females were housed individually in nesting boxes. The females and offspring were housed together in these cages through LD 22.

4. **Animal Assignment:** Mated females were randomly assigned (stratified by body weight) to test groups as shown in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 5.

**Table 1. Study design** [a]

| Experimental Parameter | Dose (ppm) | | | | Subsets |
|---|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 | |
| **Maternal Animals** | | | | | |
| No. of maternal animals assigned | 25 | 25 | 25 | 25 | NA |
| Detailed clinical observations (daily from GD 0 through LD 22) | 25 | 25 | 25 | 25 | NA |
| **Offspring** | | | | | |
| Developmental landmarks | All pups/litter | All pups/litter | All pups/litter | All pups/litter | 1, 2, 3, 4, 5 |
| Detailed clinical observations<br>(PND 5)<br>(PND 12) | 1 pup/sex/litter b<br>1 pup/sex/litter | 1 pup/sex/litter<br>1 pup/sex/litter | 1 pup/sex/litter<br>1 pup/sex/litter | 1 pup/sex/litter<br>1 pup/sex/litter | 1, 2, 3, 4, 5<br>4 |
| Motor activity<br>(PND 14, 18, 22, 62) | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 3 |
| Auditory startle habituation<br>(PND 23, 63) | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 3 |
| Passive avoidance<br>(PND 23, 24, 30, 31) | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 2 |
| Watermaze<br>(PND 62, 63, 69, 70) | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 1 pup/sex/litter | 2 |
| Brain weight<br>PND 12<br>PND 83-87 | <br>10/sex<br>10/sex | <br>10/sex<br>10/sex | <br>10/sex<br>10/sex | <br>10/sex<br>10/sex | <br>1<br>4 |
| Neuropathology<br>PND 12<br>PND 83-87 | <br>10/sex<br>10/sex | <br>10/sex<br>10/sex | <br>10/sex<br>10/sex | <br>10/sex<br>10/sex | <br>1<br>4 |

a Data obtained from MRID 45422804, pages 42-48.
b 20 pups/sex/group.
NA Not applicable

5. **Dose selection rationale:** In a previous developmental toxicity study (Argus Research #1120-001), reduced maternal body weight gain and food consumption were observed at 125 mg/kg/day (approximately 1900-2000 ppm). No developmental toxicity was observed at doses up to 125 mg/kg/day. In a two-generation reproduction study (study # not reported), decreased maternal body weight gains and pup body weights and body weight gains were observed at doses as high as 2500 ppm. No reproductive toxicity was observed at doses as high as 500 ppm. Additionally, data from a subchronic neurotoxicity screening battery (doses of 150, 1000, and 3000 ppm) showed reduced body weight and food consumption at 3000 ppm. The preliminary evaluations indicated that the 1000 ppm group was not significantly affected. Based on these studies, the doses shown in Table 1 were selected for the current study.

**6. Dosage preparation, administration, and analysis:** Test diets were prepared by the Diet Preparation Lab at Bayer Corporation, Toxicology (Stilwell, KS), and weekly aliquots were shipped refrigerated as needed to the testing facility. Weekly aliquots were stored at room temperature during use. P generation females were supplied appropriate dietary admixtures beginning on GD 0 and continuing through GD 24 (rats that did not deliver a litter) or LD 22 (rats that did deliver a litter). F1 animals were not directly supplied with the test diets. Each batch of diet admixture was sampled at Bayer Corporation prior to shipping. It was stated that homogeneity and stability of the test material in the diet were determined at 50 and 5000 ppm in previous studies. Concentration was determined for each dose formulation prepared during the study.

**Results**

**Homogeneity:** It was stated that the homogeneity of the test material in the diet was confirmed at 50 and 5000 ppm.

**Stability:** It was stated that the test material was stable in the diet for at least 7 days at room temperature or refrigerated for 28 days.

***Concentration (mean % of nominal):*** (MRID 45422804, page 738)

100 ppm = 99.3% (n=1; on GDs 0-1, 12 rats in the 150 ppm group were inadvertently offered diets of 100 ppm)
150 ppm = 92.5% (Range: 82.7-93.3%, n=4)
500 ppm = 90.7% (Range: 87.8-94.0%, n=4)
1750 ppm = 96.3% (Range: 82.6-106%, n=4)

On one occasion each for the 150 and 1750 ppm dose groups, the dietary formulations were found to be approximately 17% below nominal concentrations, which is considered somewhat lower than optimal. It appeared that there was no effort made to correct this deficiency (i.e., reformulate the dietary mixes) during the two-week periods that the formulated feeds were fed to the test animals. The impact of this formulation problem on the study results is not expected to be significant, since in each case it occurred during the final two weeks of treatment, when relative feed consumption for pups would tend to be somewhat high. Overall for the duration of test substance administration, the mean concentration of test substance in feed was within 10% of nominal, indicating that the variation between nominal and actual dosage to the study animals was generally acceptable.

## C. OBSERVATIONS

### 1. In-life observations

**a. Maternal animals:** The P animals were checked for mortality twice daily; clinical signs were recorded daily from GD 0 though LD 22. Maternal behavior of the dams was evaluated on PNDs 1, 5, 8, 14, and 22.

Dams were not subjected to a full functional observational battery (FOB). However, during treatment (GD 0 through LD 22), the dams were observed at approximately the same time each day by an individual who was unaware of each animal's dosage group. The functional

observations described below were recorded; however, the study report did not describe the procedures used for these observations, e.g., whether the same technicians were used throughout testing, where the testing was done (no mention was made as to whether animals were observed outside the home cage), when the testing was done with respect to time of dosing, the environmental conditions, whether a scoring or ranking system was used, or the duration of the observation period.

| FUNCTIONAL OBSERVATIONS | |
|---|---|
| X | Signs of autonomic function, including:<br>1) Assessment of lacrimation and salivation, and respiration<br>2) Presence or absence of piloerection,<br>3) Observations of urination and/or defecation,<br>4) Degree of palpebral closure and "prominence of the eye" |
| X | Incidence of abnormal movements. |
| X | Incidence of abnormal postures. |
| X | Incidence of abnormal behavior patterns and/or unusual appearance. |

Data taken from text, p. 42, MRID 45422804.

Body weights were measured weekly prior to treatment, daily beginning on GD 0, and at necropsy. Food consumption was recorded daily during the treatment period. Body weight gains were calculated for each corresponding gestation or lactation interval, as well as for GDs 0-20 and LDs 1-22.

P females that did not deliver a litter were sacrificed on presumed GD 25, necropsied, and examined for gross lesions and evidence of pregnancy. All other P dams were sacrificed on LD 22 and subjected to a gross pathological examination. Gross lesions were preserved in 10% neutral-buffered formalin for possible future histopathological examination.

**b. Offspring**

**1) Litter observations:** Litter size, live litter size, and pup sex and viability at birth were evaluated. For pups that died prior to the initial viability examination, the lungs were removed and immersed in water to determine whether the pup was live- or stillborn. Litters were checked for dead pups at least twice daily, and numbers of pups in each litter and clinical observations were recorded once daily preweaning. Live pups were weighed individually on PND 1, 5, 8, 12, 14, 18, and 22.

On PND 5, litters were standardized using a table of random units to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible), and the excess pups were killed and necropsied. Litters with fewer than 9 pups were retained until PND 12, at which time the offspring were assigned to Subsets 1-5, as previously described

**2) Developmental landmarks:** Pups were evaluated for the following physical and sexual landmarks. Testing continued until the day that all pups in the group achieved the criterion.

| Landmark | Subsets | First Day of Observation |
|---|---|---|
| Surface righting reflex (ability to right in 5 seconds) | 1-5 | PND 1 |
| Pinna unfolding | 1-5 | PND 2 |
| Eye opening | 2-5 | PND 12 |
| Acoustic startle response | 2-5 | PND 13 |
| Pupil constriction | 2-5 | Once on PND 21 |
| Vaginal patency | 2-4 females | PND 28 |
| Preputial separation | 2-4 males | PND 39 |

**3) Postweaning observations:** After weaning on PND 22, offspring were examined twice daily for mortality and morbidity. Clinical observations and body weights were recorded weekly until sacrifice. Food consumption was recorded weekly beginning on PND 30

**4) Neurobehavioral evaluations:** The offspring subsets were assigned to the following tests. The same animals were used for passive avoidance and water maze testing, and the same animals were used for motor activity and auditory startle habituation.

**i) Functional observational battery (FOB):** On PND 5, 10 randomly selected pups/sex/group (1/sex/litter from 10 litters) were examined outside the home cage (no information regarding performance of this examination was provided). On PND 12 and weekly during the postweaning period, the offspring in Subset 4 were examined; examination outside the home cage was specified for PND 12 only. Offspring were not subjected to a full FOB; however, observations were made as described above for the dams. Rats assigned to Subsets 2 and 3 were "examined for gross signs of toxicity" when they were removed from their cages for behavioral testing and/or weighed.

**ii) Motor activity testing:** Motor activity was evaluated in the pups from Subset 3 on PND 14, 18, 22, and 60; the same pups were evaluated each time. A passive infrared sensor mounted outside a stainless-steel 40.6 x 25.4 x 17.8 cm cage (with Plexiglas® flooring during preweaning) was used to record the number of movements and time spent in movement over the course of a 1-hour session, with tabulation at each 5-minute interval. A rack of up to 32 cages and sensors was monitored during each session. Each rat was tested in the same location on the rack across test sessions, and groups were counterbalanced according to sex and treatment level across testing sessions and cages, where possible. No information was provided as to whether testing was performed at the same time of day across sessions.

**iii) Auditory startle reflex habituation:** Auditory startle reflex habituation testing was evaluated in the pups from Subset 3 on PND 23 and 63, using a microcomputer to control the test

session. Testing was conducted in a sound-attenuated chamber, using sets of 4 rats per session. Each rat was placed in a small cage above a platform that contained a force transducer in its base. There was an initial adaptation period of 5 minutes, and during the last minute of this period 10 "blank" trials were given to sample the baseline force in the absence of a stimulus. The rats were then given 50 trials of 30 msec, 120 dB bursts of noise at 10-second intervals, followed by an additional 10 "blank" trials. The microcomputer sampled the output of the force transducer and recorded the peak amplitude of each response. The response magnitude was calculated by subtracting the average response on baseline trials, and the average response magnitude and the pattern of responses over 10-trial blocks were compared among treatment groups.

iv) **Learning and memory testing:** Learning and memory testing was performed on animals in Subset 2 (1 pup/sex/litter/dose group).

Passive avoidance test: A passive avoidance test was conducted on PNDs 23 or 24 and again seven days later to assess learning, short-term retention, long-term retention, and hyperactivity; each animal was tested twice, with a one-week interval between test sessions. For each trial, the animal was placed in the "bright" compartment of a two-compartment chamber, the sliding door between compartments was opened, and the light was turned on. When the animal entered the "dark" compartment, the sliding door was closed, the light was turned off, and a 1 second pulse of 1 mA electric current was delivered to the grid floor of the compartment. The animal was then removed from the apparatus and placed in a holding cage for 30 seconds before the start of the next trial. The criterion for learning was that the rat remained in the "bright" compartment for 60 seconds on two consecutive trials, and trials were repeated until the criterion had been met or until 15 trials had been completed. For each trial the latency to enter the dark compartment was recorded.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the latency to enter the "dark" compartment on trial 1 of the first test session (activity levels and exploratory tendencies in a new environment); the latency to enter the "dark" compartment on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); and the latency to enter the "dark" compartment on trial 1 of the second session (long-term retention).

Water maze: Water maze testing was conducted on PND 62 and 63 and again seven days later to assess overt coordination, swimming ability, learning, and memory. Testing was conducted using a watertight, 16-gauge stainless-steel modified M-maze filled with 21±1°C water at a depth of approximately nine inches, and each animal was tested twice, with a one-week interval between test sessions. For each trial, the rat was placed in the starting position at the base of the M-maze, farthest from the two arms and required to swim to one of the two goals to be removed from the water. On the initial trial, the rat had to enter both arms of the maze before being removed from the water, and the first arm chosen was designated as the incorrect goal during the remaining trials of both test sessions. For each trial, the animals were given 60 seconds to make a correct goal choice, and animals failing to make a correct choice within that time were guided to the correct goal and then removed from the water. The inter-trial interval was 15 seconds.

The criterion for learning was five consecutive errorless trials, and trials were repeated with a 15-second inter-trial interval until the criterion had been met or until 15 trials had been completed. For each trial, the latency to choose the correct goal and the number of errors, i.e., incorrect turns in the maze, were recorded. No information was provided regarding criteria for scoring errors.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the average number of errors for each trial on the first day of testing (for overall learning performance); the latency to reach the correct goal on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); the average number of errors for each trial in the second session (long-term retention); and the latency to reach the correct goal on trial 1 of the second session (long-term retention).

**2. Postmortem observations**

**a. Maternal animals:** Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 25 (rats that did not deliver) or LD 22 (following weaning of their litter) and subjected to gross necropsy of the thoracic, abdominal, and pelvic cavities. The number and distribution of implantation sites was recorded, and tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination. Additionally, dams with no surviving pups were sacrificed after the last pup was found dead or missing and presumed cannibalized. Postpartum data for these dams were excluded from summary tables.

**b. Offspring:** Pups that died prior to litter examinations for pup viability were evaluated for vital status at birth, as previously described. Gross necropsies were conducted on all pups found dead or sacrificed moribund, as well as the pups that were culled on PND 5, the pups from Subset 5 not used as replacement animals (sacrificed on PND 22), and the animals from Subset 4 that were not selected for neuropathological examination (sacrificed on PND 83-87). All pups were sacrificed by carbon dioxide asphyxiation, except for those in Subset 4, which were sacrificed by overdose of sodium pentobarbital. For necropsies conducted on pups dying or sacrificed on or before PND 5, pups with gross lesions were preserved in Bouin's solution. For necropsies conducted after PND 5, gross lesions were preserved in 10% neutral buffered formalin for possible future evaluation. All gross lesions were subjected to histological examination.

The offspring selected for brain weight or neuropathological evaluation were sacrificed on PNDs 12 (subset 1) or 83-87 (subset 4). These animals were subjected to postmortem examinations as described below.

**On postnatal day 12,** the approximately twenty pups/sex/group of Subset 1 were sacrificed by carbon dioxide asphyxiation and subjected to gross necropsy. The head of each pup was severed just behind the back of the skull and the calvarium was removed from the top of each skull prior to immersion fixation of the entire head in 10% neutral buffered formalin. The heads were then sent to Consultants in Veterinary Pathology, Inc. (Murrysville, PA) for additional processing and evaluation. Upon arrival, the brains were removed and weighed, and 10 undamaged brains/sex/group were randomly selected for microscopic evaluation. Prior to sectioning, the

following gross measurements were taken (in a blinded manner) using a Vernier caliper: the anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and the AP length of the cerebellum, extending from the anterior edge of the cortex to the posterior. The brains were then cut into six coronal slices, by means of the following cuts: 1) half-way between the ventral base of the olfactory bulbs and the optic chiasm; 2) through the optic chiasm; 3) through the infundibulum; 4) through the midbrain just posterior to the mammillary body; 5) through the cerebellum just anterior to its midpoint; and 6) through the anterior portion of the medulla. The tissues were embedded in paraffin, sectioned at 7μ, and stained with hematoxylin and eosin, and histopathological examination was performed on tissues from control and high-dose pups. In addition, the following microscopic measurements were taken (in a blinded manner), using a calibrated, ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex (the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm), 3) diagonal width of the caudate putamen and underlying globus pallidus within the coronal section taken at the level of the optic chiasm, 4) thickness of the corpus callosum within the section taken at the level of the optic chiasm, 5) thickness of the dorsal portion of the dentate gyrus of the hippocampus within the section taken at the level of the infundibulum; 6) the maximum height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface, and 7) the thickness of the external germinal layer of the cerebellum (measurements were taken from six areas over the dorsum of the cerebellum, and the mean value was reported). For those areas measured bilaterally, only the mean was provided in the data report.

**On postnatal days 83-87,** the 10 animals/sex/group from Subset 4 selected for neurohistological evaluation were sacrificed by administration o
f heparin and sodium pentobarbital, perfused *in situ* with 10% neutral buffered formalin, and subjected to gross necropsy. The head of each animal was severed between the back of the skull and the first cervical vertebra, and the calvarium was removed from the top of each skull prior to immersion of the entire head in 10% neutral buffered formalin for additional fixation. The dorsal arches of the vertebrae were removed to expose the spinal cord, and the hind limbs were dissected to expose the peripheral nerves. The spinal column and legs were placed in neutral buffered formalin for additional fixation. All tissues were sent to Consultants in Veterinary Pathology, Inc. (CVP, Murrysville, PA); neural tissues other than the brain were forwarded to Experimental Pathology Laboratories, Inc. (Herndon, VA) for processing and examination.

The brains were processed and evaluated as follows. Upon arrival at CVP, the brains were removed and weighed, and 10 brains/sex/group were selected for microscopic evaluation. Prior to sectioning, the following gross measurements were taken (in a blinded manner) using a Vernier caliper: the anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and the AP length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole (uvula) (this measurement was taken on the diagonal). The brains were then cut into eleven coronal slices approximately 2-3 mm in thickness, by means of the following cuts: 1) just posterior to the olfactory bulbs; 2) midway between the optic chiasm and the plane of the first section; 3) just anterior to the optic

chiasm; 4) through the median eminence just anterior to the infundibulum; 5) just anterior to the posterior edge of the mammillary body; 6) immediately in front of the anterior edge of the pons; 7) just anterior to the middle of the cerebellar cortex; 8) through the posterior portion of the cerebellar cortex; and 9) through the anterior portion of the medulla. The brain tissues and gasserian ganglia and associated trigeminal nerve were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, luxol fast blue/cresyl violet, and the Bielschowsky's technique. Histopathological examination was performed on tissues from control and high-dose animals. In addition, the following microscopic measurements were taken (in a blinded manner), using a calibrated, ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex (the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm), 3) diagonal width of the caudate putamen and underlying globus pallidus within the coronal section taken at the level of the optic chiasm, 4) thickness of the corpus callosum within the section taken at the level of the optic chiasm, 5) thickness of the dorsal portion of the dentate gyrus of the hippocampus within the section taken at the level of the mamillary body; and 6) the maximum height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface. For those areas measured bilaterally, only the mean was provided in the data report.

Peripheral nervous system tissues were processed as follows at Experimental Pathology Laboratories, Inc., Herndon, VA. The Gasserian ganglia and associated trigeminal nerve tissue were removed from the floor of the cranial vault. After remaining portions of the dorsal arches were trimmed away, the cord was lifted out of the spinal canal with the spinal nerve roots and ganglia attached. The dorsal and ventral nerve roots (including the dorsal root ganglia) were then removed, and sections were taken from the cervical and lumbar swellings, the mid-thoracic cord, and the cervical cord. The sciatic nerve and its branches were removed from one hind leg; sections of the sciatic, tibial, common peroneal (fibular) and sural nerves were taken.

The following central and peripheral nervous tissues (X) were dissected, preserved in paraffin (CNS tissues) or glycol methacrylate (PNS tissues), blocked, sectioned, and stained with hematoxylin and eosin, Bielschowsky's technique, and luxol fast blue/cresyl violet (paraffin tissue blocks, 5 micrometer sections) or hematoxylin and eosin, Bielschowsky's technique, and toluidine blue (glycol methacrylate blocks, 2 micrometer sections). Neurohistological evaluation was performed on tissues from males and females in the control and high dose groups.

The following CHECKED (X) tissues from the control and 1750 ppm groups, as well as the 500 ppm females were examined microscopically:

| X | CENTRAL NERVOUS SYSTEM | X | PERIPHERAL NERVOUS SYSTEM |
|---|---|---|---|
| | **BRAIN** | | **PERIPHERAL NERVES** |
| X | Olfactory bulbs | X | Sciatic (cross- and longitudinal sections) |
| X | Cerebral cortex | X | Tibial (cross- and longitudinal sections) |
| X | Hippocampus | X | Common peroneal (longitudinal section) |
| X | Basal ganglia | X | Sural (longitudinal section) |
| X | Thalamus | | |
| X | Hypothalamus | | |
| X | Midbrain | | |
| X | Cerebellum | | |
| X | Pons | | |
| X | Medulla oblongata | | |
| | **SPINAL CORD** (Cross and longitudinal sections) | | **OTHER** |
| X | Cervical | X | Dorsal root ganglia (longitudinal sections) |
| X | Thoracic | X | Spinal nerve roots (longitudinal sections) |
| X | Lumbar | | |
| | **OTHER** | | |
| X | Gasserian ganglion | | |
| X | Trigeminal nerves | | |

Data taken from Appendix M, pp. 754-756 and 808-811, MRID 45422804.

## D. DATA ANALYSIS

**1. Statistical analyses:** In general, continuous data (body weight, food consumption, latency and errors per trial scores in behavioral tests, and percent mortality per litter) were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures; either a Kruskal-Wallis test followed by Dunn's test (if ≤75% of the scores were tied) or Fisher's Exact test (if >75% of the scores were tied).

Motor activity and auditory startle habituation interval data were analyzed using a repeated measures ANOVA for a Dosage effect or a Dosage x Block interaction. If the Dosage effect was significant the data were analyzed using Dunnett's test. If the Dosage x Block interaction was

significant, the data were analyzed further using ANOVA, followed by Dunnett's test as necessary.

Litter size, number of trials to criterion, and developmental landmark data were analyzed using non-parametric procedures; either a Kruskal-Wallis test followed by Dunn's test (if ≤75% of the scores were tied) or Fisher's Exact test (if >75% of the scores were tied).

Clinical observations and other proportion data were analyzed as contingency tables using Variance Test for Homogeneity of the Binomial Distribution. The level of significance was set at $p \leq 0.05$ for all tests.

**2. Indices:** The following indices were calculated:

**a. Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Gestation index = (Number of animals with live offspring/number of pregnant animals) x 100

**b. Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study:

Viability index (%) = (# live pups on PND 5 precull/# live pups on PND 1) x 100

LD 5-12 Lactation index (%) = (# live pups on PND 12/# live pups on PND 5 postcull) x 100

LD 12-22 Lactation index (%) = (# live pups on PND 22/# live pups on PND 12) x 100, where the number of live pups on PND 22 excludes those not selected for further evaluation, and the number of live pups on PND 12 excludes those sacrificed for fixed brain weights and/or neurohistological evaluations.

**3. Positive and historical control data:**

A number of positive control study reports were included in an appendix (pp. 959-1412) to the clothianidin study report (se Appendix A to this DER for a detailed summary), and included functional observational battery, motor activity, auditory startle, passive avoidance, water maze, and subjective and morphometric neuropathology assessments following exposures to known neurotoxic agents. Some of these studies were unacceptable for use to support the current study since they predated the current study by nearly a decade and used procedures and technical staff that are no longer current to the performing laboratory or relevant to the current study. On the other hand, there were some studies in the package that were conducted within the past 5 years, that focused on the assessment of young animals, and were able to demonstrate the ability of the performing laboratories (Argus Laboratory and Consultants in Veterinary Pathology, Inc.) to detect adverse neurobehavioral and/or neuropathological effects of known neurotoxic agents. While the positive control data submission is not optimal, it does provide a certain degree of confidence in the performance of the DNT study with clothianidin.

Historical control data were provided for standard functional observational battery parameters, developmental landmarks, motor activity, passive avoidance, auditory startle habituation, water maze, brain weights, and brain morphometrics (pp. 860-958). Some of these data were derived from different procedures than those used in the clothianidin study (e.g., FOB and motor activity).

## II. RESULTS

### A. PARENTAL ANIMALS

1. **Mortality, clinical signs, and functional observations:** All dams survived until scheduled termination. No treatment-related findings were observed during the conduct of detailed clinical observations. One high-dose dam exhibited limited use of the right forelimb during GD 1-7; no other abnormal autonomic functions were reported in any group. Incidental findings of localized alopecia, abrasions, or scabs on the extremities were observed in all groups. Soft or liquid feces occurred in one low-dose dam on LD 11-13 and in one high-dose dam on LD 12; however, normal autonomic function was consistently reported for these dams.

2. **Body weight and food consumption:** Body weights and body weight gains for the P females are presented in Tables 3a and 3b; food consumption values are summarized in Table 4. At 1750 ppm, body weights were consistently decreased ($p \leq 0.05$ or not statistically significant [NS]) throughout gestation and lactation (↓2-8%). Body weight gains were decreased ($p \leq 0.05$) during GDs 0-3 (↓63%) and LDs 4-7 (↓67%). These decreases corresponded with the reductions ($p \leq 0.05$) noted in absolute (↓7-23%) and relative (↓6-22%) food consumption during the gestation and lactation periods (Table 4). Body weight gains increased ($p \leq 0.05$) during LDs 14-22 at 500 (↑162%) and 1750 (↑215%) ppm; and overall body weight gains were not significantly different from controls for the gestation (GD 0-20) and lactation (LD 1-22) periods.

**Table 3a. Selected mean (± SD) body weights (g) for P females administered TI 435 from GD 0 to LD 22. [a]**

| Treatment interval (days) | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Gestation** | | | | |
| 0 | 231.0±11.3 | 232.0±11.1 | 231.3±11.6 | 232.4±10.5 |
| 7 | 259.6±12.7 | 260.0±11.7 | 259.5±14.9 | 250.3±11.7* (↓4) |
| 15 | 305.0±14.5 | 306.4±13.9 | 307.8±20.0 | 295.4±17.3 |
| 17 | 327.8±16.3 | 329.5±17.0 | 332.0±21.7 | 316.4±19.2* (↓3) |
| 20 | 367.2±20.8 | 374.0±21.0 | 376.3±26.5 | 356.7±22.8 |
| **Lactation** | | | | |
| 1 | 280.3±12.7 | 280.4±15.6 | 278.7±20.1 | 267.1±15.2** (↓5) |
| 4 | 277.3±21.3 | 283.8±20.2 | 280.4±22.4 | 272.6±13.5 |
| 7 | 294.1±16.0 | 289.7±16.5 | 290.7±21.7 | 279.2±17.9** (↓5)[b] |
| 14 | 319.2±21.6 | 318.5±18.1 | 309.4±25.4 | 294.4±20.3** (↓8) |
| 19 | 334.0±16.4 | 339.6±21.6 | 334.2±21.7 | 319.2±24.6* (↓4) |
| 22 | 330.0±28.9 | 337.8±16.2 | 337.7±16.2 | 328.4±21.3 |

a Data were obtained from MRID 45422804, Tables B3 and B5, pages 95, 96, 98, and 99. Percent difference from controls is presented parenthetically; n= 23-25.
b Excludes values from dams that had fewer than 9 pups on LD 5; n=22.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

**Table 3b. Selected mean (± SD) body weight gains (g) for P females administered TI 435 from GD 0 to LD 22 [a]**

| Treatment interval (days) | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Gestation** | | | | |
| 0-3 | 13.9±6.5 | 11.6±8.7 | 12.8±7.6 | 5.1±6.8** (↓63) |
| 3-6 | 12.3±7.7 | 14.9±6.9 | 14.1±5.6 | 13.0±6.8 |
| 18-20 | 25.4±14.1 | 28.4±6.8 | 28.8±5.9 | 26.1±5.0 |
| Overall (0-20) | 136.2±21.7 | 142.0±20.7 | 145.1±18.0 | 124.3±18.0 |
| **Lactation** | | | | |
| 1-4 | 3.0±22.0 | 3.5±12.9 | 1.7±17.2 | 5.5±11.6 |
| 4-7 | 16.4±17.5 | 5.8±8.6* (↓65) | 10.3±15.5 | 5.4±12.2* (↓67)[b] |
| 14-22 | 10.8±25.5 | 19.4±13.7 | 28.3±15.1* (↑162)[c, d] | 34.0±17.3** (↑215)[c] |
| Overall (1-22) | 51.6±30.8 | 57.4±9.0 | 62.1±10.1 | 62.4±14.8 |

a Data were extracted from MRID 45422804, Tables B4 and B6, pages 97 and 100. Percent difference from controls is presented parenthetically; n=23-25.
b Excludes values from dams that had fewer than 9 pups on LD 5; n=22.
c Excludes values from dams that were not selected for continued observation; n=20.
d Excludes values from dam 5550, which had no surviving pups on LD 10; n=20.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

When compared to concurrent controls, absolute (g/animal/day) food consumption (Table 4) was reduced ($p \leq 0.05$) in the 1750 ppm dams during GDs 0-9 (↓11-23%), 15-20 (↓7-8%), and 0-20 (↓10%) and LDs 7-22 (↓10%). Relative (g/kg/day) food consumption was reduced ($p \leq 0.01$) in the 1750 ppm dams during GDs 0-9 (↓8-22%) and 0-20 (↓7%), and LDs 14-22 (↓7%, $p \leq 0.05$) and 1-22 (↓6%, $p \leq 0.05$). In addition, relative food consumption was decreased ($p \leq 0.05$) at 500 ppm during GDs 0-3 (↓7%).

**Table 4. Selected mean (± SD) absolute (g/animal/day) and relative (g/kg/day) food consumption for P females administered TI 435 from GD 0 to LD 22**[a]

| Treatment interval (days) | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| **Gestation** | | | | | |
| Absolute | 0-3 | 19.6±2.2 | 19.2±3.0 | 18.2±2.3 | 15.1±1.9** (↓23) |
| | 3-6 | 21.6±2.6 | 21.8±2.2 | 21.7±2.2 | 18.9±2.6** (↓13) |
| | 6-9 | 24.4±2.2 | 23.9±2.8 | 23.7±2.6 | 21.7±2.6** (↓11) |
| | 15-18 | 29.4±2.4 | 29.1±2.4 | 30.0±2.6 | 27.4±2.8* (↓7) |
| | 18-20 | 27.0±3.1 | 27.4±2.7 | 27.6±2.8 | 24.8±2.4* (↓8) |
| | Overall (0-20) | 24.9±1.6 | 24.6±1.8 | 24.5±2.0 | 22.4±1.6** (↓10) |
| Relative | 0-3 | 82.2±7.6 | 80.5±11.0 | 76.6±8.0* (↓7) | 64.5±6.8** (↓22) |
| | 3-6 | 85.9±9.7 | 87.0±8.4 | 86.4±6.6 | 77.3±8.8** (↓10) |
| | 6-9 | 92.5±5.3 | 90.8±9.2 | 89.8±7.6 | 85.4±8.4** (↓8) |
| | Overall (0-20) | 87.6±3.6 | 86.1±4.9 | 85.8±4.3 | 81.2±3.4** (↓7) |
| **Lactation** | | | | | |
| Absolute | 1-4 | 29.3±7.3 | 29.0±6.1 | 29.4±5.0 | 26.9±4.7 |
| | 7-14 | 55.1±6.4 | 55.2±5.4 | 52.4±5.3 | 49.4±6.1** (↓10)[b] |
| | 14-22 | 70.3±5.2 | 71.7±6.9 | 69.5±5.9 | 63.1±6.4** (↓10)[b] |
| | Overall (1-22) | 55.7±4.7 | 56.4±4.5 | 54.8±4.2 | 50.3±5.0** (↓10)[b] |
| Relative | 1-4 | 104.7±25.2 | 102.5±19.3 | 104.7±16.5 | 100.3±16.2 |
| | 7-14 | 179.8±15.8 | 180.2±12.9 | 175.7±17.7 | 169.8±16.4 |
| | 14-22 | 215.0±11.5 | 215.8±20.0 | 213.3±17.1 | 200.6±16.5* (↓7)[b] |
| | Overall (1-22) | 180.9±11.1 | 181.8±12.0 | 180.0±13.0 | 170.8±12.9* (↓6)[b] |

a Data were extracted from MRID 45422804 Tables B7-B10, pages 101-104. Percent difference from controls is listed parenthetically; n=23-25.

b Excludes values from dams that were not selected for continued observation; n=20.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

**3. Test substance intake:** Mean compound intake (mg/kg bw/day) during the gestation and lactation periods was determined based on maternal food consumption and body weight (Table 5). The investigators stated that it was presumed that pups began to consume maternal feed after

approximately LD 14. The overall average lactation test substance intake values are similar to average intake values for LD 7-14 (i.e., based upon maternal intake alone).

**Table 5. Mean (±SD) test substance intake (mg/kg/day) for P females administered TI 435 from GD 0 to LD 22** [a]

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| GD 0-20 | 0.0±0.0 | 12.9±0.7 | 42.9±2.1 | 142±6.0 |
| LD 1-22[b] | 0.0±0.0[c] | 27.3±1.8[c] | 90.0±6.5[c, d] | 299.0±22.5[c] |

a Data were extracted from MRID 45422804 Table B1, pages 92-93; n=23-25.
b It was presumed that the pups began to consume maternal feed after approximately LD 14.
c Excludes values from dams that were not selected for continued observation; n=19-20.
d Excludes values from dam 5550, which had no surviving pups on LD 10; n=20.

**4. Reproductive performance:** Pregnancy rate, number of implantations/dam, gestation length, and sex ratio were comparable between treated and control animals (Table 6). The gestation index was 100% for all groups.

**Table 6. Delivery observations in P females administered TI 435 from GD 0 to LD 22** [a]

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| # Animals Mated | 25 | 25 | 25 | 25 |
| # Animals Pregnant<br>Pregnancy Rate (%) | 25<br>(100) | 25<br>(100) | 23<br>(92) | 23<br>(92) |
| # Nonpregnant | 0 | 0 | 2 | 2 |
| Mean (±SD) gestation length (days) | 22.6±0.5 | 22.6±0.5 | 22.6±0.5 | 22.9±0.3 |
| Total # Implantations<br>Mean (±SD) Implantations/Delivered Litter | 366<br>14.6±2.0 | 380<br>15.2±2.0 | 355<br>15.4±1.6 | 347<br>15.1±1.8 |
| Total # of Litters Examined | 25 | 25 | 23 | 23 |
| Sex Ratio (% Male, ±SD)[b] | 44.4±15.9[c] | 45.9±13.8 | 50.1±16.7 | 50.9±11.1 |

a Data extracted from MRID 45422804 Tables B11 and B12, pages 105 and 109.
b Includes pups born alive, found dead on PND 1.
c Excludes values for litters 5504 and 5524; the dams delivered one additional pup each on LD 2; n=23.

**5. Maternal postmortem results**

**a. Macroscopic examination:** No treatment-related pathological abnormalities were observed in any treated group.

**b. Microscopic examination:** Microscopic examinations were not conducted on adult animals.

## B. OFFSPRING

**1. Viability and clinical signs:** No treatment-related differences in live litter size, postnatal survival, or sex ratios were observed in any treated group through PND 22 (Table 7). At 1750 ppm, two male and three female rats were found dead on PNDs 25-27 post-weaning. The study report stated that this finding was considered related to a failure to thrive post-weaning, based on the patterns of body weight gains and losses, and was related to treatment. The lactation index was decreased ($p \leq 0.05$) in the 150 and 500 ppm animals (92.7-94.8% treated vs 99.6% controls); however, this finding was considered unrelated to treatment because it was not dose-dependent and the lactation index at 1750 ppm was comparable to controls. The majority of pup deaths that contributed to the decreased lactation index at 150 and 500 ppm occurred between PND 9-12; at 150 ppm, 5 of the 18 pup deaths were observed in two litters and at 500 ppm, 9 of the 10 pup deaths were observed in one litter. No treatment-related clinical signs were observed in pups.

An apparent increase in the number of pups and litters with stillborn pups was noted at 500 and 1750 ppm (Table 7). Although a dose-response relationship was not observed (the number of stillborn pups and litters with stillborn pups was greater at 500 ppm than at 1750 ppm), this finding is considered noteworthy since an increase of stillborn pups was found in the reproduction study with clothianidin.

**Table 7. $F_1$ live litter size and survival** [a]

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Number of litters | 25 | 25 | 23 | 23 |
| Mean live litter size (pups/litter) | | | | |
| PND 1 | 13.5±2.2 [d] | 14.1±2.1 | 14.2±1.7 | 13.8±2.3 |
| PND 5 [b] | 13.2±2.0 | 13.8±2.2 | 13.6±1.8 | 13.3±2.3 |
| PND 5 [c] | 10.0±0.2 [g] | 9.9±0.3 | 10.0±0.0 | 10.0±0.0 [g] |
| PND 8 | 10.0±0.3 [g] | 9.9±0.3 | 9.9±0.3 | 10.0±0.0 [g] |
| PND 12 | 8.3±0.8 [g] | 7.6±1.7 | 7.8±1.8 | 8.2±0.6 [g] |
| PND 14 | 8.0±0.2 [h] | 7.8±0.5 [h] | 8.1±0.3 [h] | 8.0±0.2 [h] |
| PND 18 | 8.0±0.2 [h] | 7.7±0.7 [h] | 8.0±0.4 [h] | 7.9±0.3 [h] |
| PND 22 | 8.0±0.2 [h] | 7.7±0.7 [h] | 8.0±0.4 [h] | 7.8±0.5 [h] |
| No. pup deaths (litters) | | | | |
| Stillborn | 1 | 1 | 6 (5) | 3 (3) |
| PND 1 | 2 (2) | 1 | 2 (2) | 2 (2) |
| PND 2-5 | 11 (9) | 7 (6) | 12 (10) | 11 (8) |
| PND 6-8 | 1 | 0 | 2 (2) | 0 |
| PND 9-12 | 0 | 18**(4) | 10*(2) | 0 |
| PND 13-14 | 0 | 2 (2) | 0 | 1 |
| PND 15-22 | 0 | 2 (2) | 1 | 3 (2) |
| Total preweaning deaths | 15 | 31 | 33 | 20 |
| No. postweaning deaths (PND 25-27) | 0 | 0 | 0 | 5 |
| Sex ratio (% male) | 44.4±15.9 [d] | 45.9±13.8 | 50.1±16.7 | 50.9±11.1 |
| Viability index (%) | 96.1 | 97.7 | 95.7 | 95.9 |
| Lactation index (%) [e] | 99.6 | 92.7** | 94.8* | 100.0 |
| Lactation index (%) [f] | 100.0 | 97.4 | 99.4 | 97.5 |

a Data extracted from MRID 45422804, Table B12, pages 106-111, and Table B22, pages 159-166.
b Before standardization (culling).
c After standardization (culling).
d Excludes values for litters 5504 and 5524; the dams delivered one additional pup each on LD 2.
e Number of live pups in Subsets 1-5 on PND 12/ number of live pups on PND 5 post-culling. Excludes values for litters with fewer than 9 pups on LD 5.
f Number of live pups on PND 22 in Subsets 2-5 on PND 12/ number of live pups in Subsets 2-5 on PND 12. Excludes values for litters that were not selected for continued observation and pups in Subset 1.
g Excludes values for litters with fewer than 9 pups on LD 5.
h Excludes values for litters that were not selected for continued observation.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

**2. Offspring body weight and food consumption:** Selected pre- and post-weaning body weights and body weight gains for $F_1$ pups are presented in Tables 8a and 8b. At 1750 ppm, pre-weaning body weights were decreased ($p \leq 0.05$) in the males on PNDs 12-22 (↓6-16%) and in the females on PNDs 14-22 (↓13-16%). Additionally, pre-weaning body weights were decreased ($p \leq 0.05$) in the 500 ppm females on PNDs 14-22 (↓6-7%). At 1750 ppm, post-weaning body

weights were sporadically decreased ($p \leq 0.05$) in the males on PNDs 23-27 (↓4-15%) and in the females on PNDs 23-30 (↓6-13%). Body weight gains were decreased ($p \leq 0.05$) during pre-weaning between PNDs 12-22 in the males (↓11-44%) and females (↓11-48%). Overall pre-weaning (PND 5-22) body weight gains were decreased by 18% in both sexes compared to controls. In the 500 ppm females, body weight gains were decreased ($p \leq 0.05$) during PNDs 12-18 (↓8-24%) and overall pre-weaning weight gain was decreased ($p \leq 0.05$) by 7% compared to controls. Post-weaning body weight gains in the 1750 ppm females were decreased (↓21%, $p \leq 0.05$) on PNDs 65-72. Post weaning body weights in the 1750 ppm males were similar to controls. Overall (PNDs 5-79) weight gains were similar between treated animals and controls.

**Table 8a. Mean (± SD) $F_1$ pup body weights (g) [a]**

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Males** | | | | |
| Pre-weaning | | | | |
| 5 | 9.1±1.1 | 9.1±1.5 | 9.0±1.0 | 8.9±1.0 |
| 12 | 18.6±3.6 | 19.0±3.3 | 18.4±3.1 | 17.4±3.0* (↓6) |
| 18[b] | 31.6±4.4 | 32.0±5.0 | 30.3±4.5 | 26.5±4.4** (↓16) |
| 22[b] | 41.6±6.1 | 42.8±6.5 | 40.5±5.6 | 35.3±5.4** (↓15) |
| Post-weaning | | | | |
| 23[b] | 44.0±6.8 | 45.2±8.0 | 42.8±6.6 | 37.4±6.5** (↓15) |
| 30[b] | 79.7±10.3 | 82.3±13.7 | 75.4±12.7 | 72.9±12.0** (↓8) |
| 37[b] | 137.6±16.9 | 142.9±19.3 | 135.6±16.4 | 130.3±17.2* (↓5) |
| 44[b] | 196.4±20.4 | 203.8±22.7 | 195.3±19.5 | 188.9±21.6 |
| 72[b] | 381.2±30.8 | 392.7±32.6 | 379.6±30.6 | 367.6±28.0* (↓4) |
| 79[b] | 409.4±33.8 | 412.4±29.7 | 414.3±36.8 | 401.6±31.2 |
| **Females** | | | | |
| Pre-weaning | | | | |
| 5 | 8.5±1.1 | 8.8±1.4 | 8.3±0.9 | 8.4±1.0 |
| 14[b] | 22.0±3.8 | 22.1±3.6 | 20.5±3.6* (↓7) | 19.2±4.2** (↓13) |
| 18[b] | 30.8±3.8 | 30.8±4.2 | 28.6±4.3** (↓7) | 25.9±5.3** (↓16) |
| 22[b] | 40.3±5.3 | 41.4±5.2 | 38.0±5.5* (↓6) | 34.4±6.3** (↓15) |
| Post-weaning | | | | |
| 23[b] | 42.1±5.6 | 43.9±6.9 | 40.3±6.4 | 36.5±7.6** (↓13) |
| 30[b] | 73.9±8.1 | 77.2±10.0 | 69.5±11.5 | 69.6±9.0* (↓6) |
| 37[b] | 118.6±11.1 | 123.4±12.3 | 114.5±14.9 | 115.1±12.1 (↓3) |
| 44[b] | 150.6±16.0 | 155.0±18.8 | 147.1±16.6 | 148.0±13.5 |
| 79[b] | 246.7±24.0 | 249.9±23.8 | 246.9±32.2 | 248.0±19.0 |

a Data obtained from MRID 45422804, Tables C3 and C5, pages 268-269 and 272-273. Percent difference from controls is presented parenthetically; n = 56-80 through PND 12

b Excludes values for rats from Subset 1, these rats were sacrificed on PND 12; n=56-60

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

**Table 8b. Selected $F_1$ pup mean (±SD) body weight gains (g) [a]**

| Postnatal Day | Dose (ppm) 0 | 150 | 500 | 1750 |
|---|---|---|---|---|
| **Males** | | | | |
| Pre-weaning | | | | |
| 5-8 | 4.0±2.7 | 4.1±1.3 | 3.9±1.2 | 3.6±1.0 |
| 12-14 [b] | 4.3±1.2 | 3.9±1.9 | 3.6±2.2 | 2.4±1.9** (↓44) |
| 14-18 [b] | 8.9±1.3 | 9.1±1.5 | 8.3±1.8* (↓7) | 6.8±1.5** (↓24) |
| 18-22 [b] | 9.9±2.5 | 10.8±2.3 | 10.2±1.7 | 8.8±1.7** (↓11) |
| 5-22 [b] | 32.5±5.4 | 33.6±6.0 | 31.4±5.1 | 26.5±5.2** (↓18) |
| Post-weaning | | | | |
| 23-79 [b] | 365.4±31.8 | 366.5±27.2 | 371.4±34.0 | 363.8±28.0 |
| (Overall) 5-79 [b] | 400.2±33.1 | 403.3±29.3 | 405.2±36.2 | 392.8±30.4 |
| **Females** | | | | |
| Pre-weaning | | | | |
| 5-8 | 3.6±1.4 | 4.0±1.4 | 3.7±1.1 | 3.4±0.9 |
| 12-14 [b] | 4.2±1.3 | 3.8±1.6 | 3.2±1.9** (↓24) | 2.2±1.9** (↓48) |
| 14-18 [b] | 8.8±1.3 | 8.7±1.2 | 8.1±1.5* (↓8) | 6.8±1.8** (↓23) |
| 18-22 [b] | 9.5±2.5 | 10.6±2.0 | 9.4±1.8 | 8.5±1.7* (↓11) |
| 5-22 [b] | 31.8±4.8 | 32.6±4.4 | 29.7±5.0* (↓7) | 26.0±5.9** (↓18) |
| Post-weaning | | | | |
| 65-72 [b] | 16.1±6.7 | 16.1±7.9 | 15.2±7.1 | 12.7±6.9* (↓21) |
| 23-79 [b] | 204.0±22.9 | 205.1±22.0 | 205.6±29.8 | 211.0±16.6 |
| (Overall) 5-79 [b] | 238.0±24.1 | 241.0±23.0 | 238.6±32.1 | 239.6±18.8 |

a Data obtained from MRID 45422804, Tables C4 and C6, pages 270-271 and 274-275. Percent difference from controls is presented parenthetically; n = 56-80 through PND 12.

b Excludes values for rats from Subset 1, these rats were sacrificed on PND 12; n=56-60

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

Absolute (g/animal/day) and relative (g/kg/day) food consumption were comparable between treated and control animals. Differences from control ($p \leq 0.05$) noted in the 1750 ppm males on PND 51-58 and 65-72 and in the 150 ppm females on PND 30-37 were considered unrelated to treatment as they were minor, transient, and not dose-dependent (MRID 45422804, Tables C7 and C9, pages 276 and 278; not presented in DER).

### 3. Developmental landmarks

**a. Sexual maturation:** No treatment-related differences in balanopreputial separation or vaginal patency were observed between treated and control $F_1$ animals (Table 9a). A slight delay in vaginal patency (35 days treated vs. 34 days controls, $p \leq 0.01$) was observed in the 500 ppm females; however, it was not dose-dependent and considered unrelated to treatment.

**Table 9a. Sexual maturation (mean days ± SD) in $F_1$ generation rats [a]**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| N (M/F) | 60/60 | 56/56 | 60/60 | 58/57 |
| Preputial separation (Males) | 47.1±3.0 | 46.8±3.0 | 47.7±2.7 | 48.3±3.5 |
| Vaginal patency (Females) | 34.0±1.4 | 33.7±1.2 | 35.0±1.7** | 34.1±1.3 |

a Data extracted from MRID 45422804, Table C11, page 280.
** Significantly different from controls at $p \leq 0.01$

**b. Physical landmarks:** No treatment-related effects on pinna unfolding (examined on PND 2-6), acoustic startle response (examined on PND 13-19), eye opening (examined on PND 12-20), or pupil constriction (examined on PND 21) were observed (MRID 45422804, Table B14, pages 114-116; not presented in DER).

A significant delay in development of the surface righting reflex (evaluated on PND 1-11) was observed on PND 3 at 150 (↓34%), 500 (↓34%), and 1750 (↓47%) ppm compared to controls (Table 9b). Initiation of the reflex was similar for control and high dose pups at PND 1. By PND 2, the response was delayed (NS) in an apparent dose-dependant manner at 500 and 1750 ppm, with significance achieved at PND 3 for all treated groups. Delays remained on PND 4 (NS), and at PND 5, the mean percent of pups reaching the criterion was similar between treated and control groups. Historical control data from 7 studies conducted in Sprague Dawley rats at the performing laboratory (MRID 45422804, page 876) demonstrate that on PND 3 an average of 40.2±11.7 percent of the pups met the criterion for surface righting, with a minimum of 23.6% and a maximum of 52.0% on that day postpartum. This historical control value is similar to the PND 3 control value from the current study.

The average day postpartum that surface righting was observed in at least 50% of the pups was similar between control and treated litters, and the control value of 3.8 ± 2.0 (Table 9b) was similar to the historical control value of 3.3 ± 1.1. For the 1750 ppm group, this value was slightly decreased (NS) as compared to control, suggesting achievement at a slightly earlier age, a result which is not consistent with the significant delay in the mean percent of pups reaching the criterion, as described above for PND 3.

The inconsistent findings reported for this parameter can be attributed to the manner in which the endpoint is evaluated, specifically that all pups in a litter are examined on each day of assessment. In other words, pups that have shown the ability to right themselves are not excluded from testing on subsequent days. Some pups do not consistently demonstrate the reflex, which can result in erratic results within a litter. (An example is control litter 5575, which demonstrated 3, 1, 4, 11, 6, and 10 pups (of 10) with the righting reflex on PND 1 through 6, respectively.) The reason for this inconsistent response is not apparent. However, it results in lower mean numbers of treated pups with the righting reflex on PND 3 than were observed to have the same reflex on PND 2. Positive control data, demonstrating the ability of this testing

paradigm to identify treatment-related alterations in the development of the righting reflex, were not provided.

In summary, apparent significant delays in development of the surface righting reflex noted on PND 3 for pups at 175, 500, and 1750 ppm were not considered toxicologically meaningful.

**Table 9b. Surface righting reflex (mean % of pups meeting the criterion ±SD) in $F_1$ generation rats [a]**

| Postnatal Day | Dose (ppm) | | | | Historical Control |
|---|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 | |
| 2 | 32.8±19.6 | 32.9±22.4 | 29.2±22.1 | 27.2±21.6 | 35.1±14.5 |
| 3 | 38.2±14.9 | 25.4±24.5* (↓34) | 25.3±19.6* (↓34) | 20.1±19.2** (↓47) | 40.2±11.7 |
| 4 | 47.3 ±18.0 | 36.3±24.7 | 34.1±15.4 | 38.4±20.1 | 51.3±12.3 |
| 5 | 48.0 ±16.2 | 48.8±23.1 | 41.1±21.7 | 52.6±26.0 | 70.1±11.7 |
| Overall [b] | 3.8 ±2.0 | 3.8±2.3 | 4.4±2.1 | 3.5±2.1 | 3.3±1.1 |

a Data obtained from MRID 45422804, Table B14, page 113. Historical control data from 7 studies conducted from 1994-1999, page 878. Percent difference from controls is presented parenthetically.
b Average day postpartum that at least 50% of pups/litter had the developmental measure present.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

## 4. Behavioral assessments

**a. Functional observational battery:** A formal FOB was not conducted on the offspring. Detailed clinical observations revealed one control male with tip-toe walk on week 10 postpartum, with subsequent hyper-reactivity in weeks 11-13 and one control male with tremors on week 7 postpartum. There were no clinical findings consistent with "abnormal autonomic functions" in offspring treated with TI 435.

**b. Motor activity:** Mean total activity count and mean time spent in movement data are summarized in Tables 10a and 10b, respectively. Mean sub-session (block) data for total activity count on all days of testing, and for time spent in movement on PND 22, are summarized in Tables 11a and 11b.

There were no apparent effects of treatment in motor activity data measured on PND 14, 18, or 62. On PND 22, mean total motor activity (number of movements) was decreased (NS) in the 1750 ppm males (↓24%) and in the females at 500 ppm (↓21%) and 1750 ppm (↓10%) (Table 10b). The mean time spent in movement on PND 22 was also decreased (NS) in the 1750 ppm males (↓27%) and in the females at 500 ppm (↓24%) and 1750 ppm (↓19%) (Table 10a). Subsession data (Tables 11a and 11b) also demonstrate significant decreases ($p \leq 0.05$) in mean motor activity counts during one to four testing blocks for 1750 ppm offspring on PND 22 (↓17-32% in males and ↓27% in females) and nonsignificant decreases (↓19-37% in males) after the

fourth testing block. Subsession data for mean time spent in movement on PND 22 (not presented) show significant decreases ($p \leq 0.05$) in 1750 ppm males (↓30% to 43% during the first 15 minutes of testing) and females (↓44% in the fourth testing block) and in 500 ppm females (↓36% in the fourth testing block). Overall, interpretation of the motor activity data is complicated by the variability in the data, as demonstrated by the large coefficients of variation, particularly for PND 14, 18, and 21 offspring. However, it is noted that the decreases in motor activity counts and the time spent in movement at PND 22 for 1750 ppm male offspring are of substantial magnitude and supported by sub-session data; therefore, they are likely related to treatment. Effects noted for 500 ppm female offspring at PND 22, while nearly of the same magnitude as those observed in PND 22 males at 1750 ppm, demonstrate a lack of dose-response and an absence of significant alterations in the subsession data. Therefore, the apparent decreases in motor activity in PND 22 females at 500 ppm are not considered to be treatment-related. (It is noted that historical control data included in the study report do not contribute to the interpretation of these data since they report total mean activity values which are substantially lower than those measured in the current study.)

In general, habituation was not observed on PND 14 or 18. Some habituation was evident in both male and female offspring on PND 22 and PND 62. There did not appear to be any treatment-related differences in habituation between control and treated rats. On all of the testing days, individual motor activity data demonstrate some animals with little or no habituation.

**Table 10a. Mean (±SD) number of movements (counts) during motor activity assessment in $F_1$ pups in Subset 3** [a]

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Males** | | | | |
| 14 | 147.2±159.5 [108] | 162.7±121.0 | 218.8±164.5 | 169.0±117.4 |
| 18 | 472.0±240.8 [51] | 367.9±245.6 | 398.6±300.8 | 447.8±242.8 |
| 22 [b] | 558.5±171.9 [31] | 490.4±212.4 | 542.5±224.3 (↓3) [41] | 422.2±237.4 (↓24) [56] |
| 62 | 723.0±175.0 [24] | 709.6±128.1 | 758.8±97.1 | 707.2±140.4 (↓2) |
| **Females** | | | | |
| 14 | 219.6±128.6 [59] | 260.5±200.4 | 199.8±181.6 | 211.7±154.8 |
| 18 | 475.1±307.8 [65] | 470.5±241.8 | 476.3±317.5 | 474.6±310.4 |
| 22 [b] | 579.5±153.6 [27] | 563.9±195.1 | 457.0±229.4 (↓21) [50] | 518.7±203.2 (↓10) [39] |
| 62 | 749.4±136.5 [18] | 663.4±122.8 | 708.2±113.3 | 651.3±150.0 (↓13) |

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically. Coefficients of variance are presented in brackets.

b Historical control data for PND 21 rats (pages 892-895 of the study report), indicate total mean values of approximately 370 (241 min-513 max) for males and 349 (181 min-529 max) for females. [The values for twelve 5-minute blocks were calculated by the reviewer from mean subsession data for eighteen 5-minute blocks and may be less accurate than if they had been generated from individual animal data.]

**Table 10b. Mean (±SD) time spent in movement (seconds) during motor activity assessment in $F_1$ pups in Subset 3** [a]

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Males** | | | | |
| 14 | 114.1±171.0 | 136.6±175.4 | 190.2±170.9 | 128.6±132.0 |
| 18 | 578.6±384.1 | 513.3±502.2 | 548.2±486.8 | 548.1±330.2 |
| 22 | 801.8±328.0 | 710.2±411.9 | 773.2±364.6 (↓4) | 588.7±409.4 (↓27) |
| 62 | 1523.5±350.4 | 1586.3±306.3 | 1759.5±317.4 | 1637.0±418.7 |
| **Females** | | | | |
| 14 | 162.9±162.9 | 229.9±264.7 | 151.0±180.2 | 141.5±144.0 |
| 18 | 595.6±466.6 | 602.7±420.8 | 627.4±560.1 | 570.8±460.2 |
| 22 | 790.2±289.9 | 800.9±357.7 | 597.9±354.8 (↓24) | 639.6±319.6 (↓19) |
| 62 | 1545.2±405.1 | 1326.0±359.6 | 1497.5±397.7 (↓3) | 1454.6±440.7 (↓6) |

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically.

**Table 11a. Mean (±SD) sub-session motor activity (counts) in $F_1$ male pups in Subset 3** [a]

| Block [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 14 | 1 | 15.3±18.6 | 15.7±15.9 | 13.4±15.0 | 15.6±18.2 |
| | 2 | 14.0±18.2 | 13.6±12.9 | 14.4±15.8 | 13.8±11.1 |
| | 3 | 17.8±21.8 | 16.1±17.2 | 17.0±19.7 | 13.8±13.2 |
| | 4 | 14.6±18.7 | 18.9±18.7 | 25.0±24.2 | 15.6±16.8 |
| | 5 | 12.4±16.8 | 18.8±17.5 | 20.1±22.8 | 13.1±10.5 |
| | 6 | 9.9±15.1 | 18.5±22.1 | 18.8±19.3 | 16.4±15.0 |
| | 7 | 11.4±14.2 | 12.6±15.2 | 14.2±20.7 | 12.0±14.5 |
| | 8 | 17.8±24.2 | 13.8±17.5 | 19.4±20.2 | 14.6±12.8 |
| | 9 | 9.4±17.2 | 8.2±12.5 | 17.8±20.1 | 13.0±15.1 |
| | 10 | 7.5±15.9 | 7.3±10.5 | 20.6±25.0 | 10.0±15.5 |
| | 11 | 9.0±14.5 | 6.8±8.5 | 19.6±24.6 | 15.4±19.0 |
| | 12 | 8.2±11.2 | 12.4±18.2 | 18.4±20.7 | 15.4±19.0 |

*(Table continues next page)*

| Block [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 18 | 1 | 35.4±26.3 | 31.0±24.2 | 23.6±28.1 | 22.1±19.8 |
| | 2 | 41.8±27.2 | 36.4±22.6 | 27.8±28.4 | 33.2±25.5 |
| | 3 | 50.6±20.6 | 38.0±22.0 | 33.8±24.8 | 39.6±26.9 |
| | 4 | 48.4±29.2 | 39.7±24.4 | 34.5±29.1 | 42.2±28.3 |
| | 5 | 47.5±28.2 | 36.6±27.0 | 40.3±30.5 | 37.2±23.8 |
| | 6 | 47.3±25.5 | 32.2±29.4 | 31.8±26.2 | 42.2±24.2 |
| | 7 | 44.0±30.2 | 28.3±25.4 | 37.2±30.7 | 36.6±26.2 |
| | 8 | 37.2±25.2 | 25.7±25.6 | 39.2±33.0 | 38.0±25.0 |
| | 9 | 37.8±30.0 | 24.6±27.5 | 31.4±29.2 | 42.2±28.4 |
| | 10 | 27.6±27.3 | 23.6±22.1 | 33.8±32.5 | 38.6±27.8 |
| | 11 | 25.0±28.7 | 27.9±28.6 | 32.5±31.2 | 38.0±28.6 |
| | 12 | 29.6±29.2 | 23.9±25.0 | 32.6±31.6 | 37.8±30.0 |
| PND 22 | 1 | 64.0±9.9 | 59.2±10.5 | 62.7±14.3 | 53.0±20.1* (↓17) |
| | 2 | 56.4±14.0 | 56.3±17.1 | 55.2±15.7 | 40.2±21.1* (↓29) |
| | 3 | 55.4±14.8 | 51.6±13.1 | 49.0±25.1 | 38.2±23.1* (↓31) |
| | 4 | 54.0±15.9 | 44.2±26.0 | 50.5±20.8 | 36.9±25.9* (↓32) |
| | 5 | 45.1±22.5 | 46.7±24.0 | 43.0±23.3 | 33.9±24.4 (↓25) |
| | 6 | 45.2±19.8 | 45.2±22.3 | 46.6±24.6 | 31.8±24.1 (↓30) |
| | 7 | 43.6±23.0 | 39.0±29.2 | 44.4±28.2 | 35.4±22.9 (↓19) |
| | 8 | 43.9±24.4 | 36.3±28.1 | 41.8±27.3 | 27.8±27.5 (↓37) |
| | 9 | 44.2±23.2 | 32.9±26.4 | 42.0±26.7 | 34.0±28.5 (↓23) |
| | 10 | 32.3±26.8 | 28.8±29.5 | 35.2±26.9 | 30.4±25.6 |
| | 11 | 34.9±28.4 | 25.7±30.7 | 36.4±28.3 | 32.2±26.8 |
| | 12 | 39.4±24.2 | 24.4±31.0 | 35.6±28.1 | 28.4±28.0 |

*(Table continues next page)*

| Block [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 62 | 1 | 70.0±7.9 | 62.9±9.0 | 64.7±10.2 | 61.4±10.1* (↓12) |
| | 2 | 67.6±9.1 | 66.4±9.5 | 65.2±7.2 | 64.0±11.3 |
| | 3 | 67.8±6.8 | 66.0±8.9 | 66.7±7.6 | 64.7±11.5 |
| | 4 | 69.2±8.0 | 65.6±11.7 | 63.8±9.6 | 60.1±9.1* (↓13) |
| | 5 | 63.0±17.9 | 67.0±10.1 | 66.0±6.7 | 62.4±14.2 |
| | 6 | 61.8±19.0 | 63.2±19.7 | 64.4±12.3 | 57.9±18.6 |
| | 7 | 61.1±21.2 | 61.2±19.1 | 63.9±10.9 | 59.1±14.2 |
| | 8 | 56.6±20.1 | 55.5±20.9 | 65.8±10.8 | 60.3±16.5 |
| | 9 | 55.2±25.1 | 57.2±23.6 | 61.0±17.3 | 57.6±21.2 |
| | 10 | 52.0±27.9 | 54.3±21.3 | 62.2±18.5 | 57.2±19.1 |
| | 11 | 53.0±31.2 | 47.2±24.7 | 55.3±20.2 | 53.3±23.6 |
| | 12 | 45.8±28.4 | 43.0±27.8 | 59.9±19.6 | 49.0±22.6 |

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically.
b Each block is a 5-minute period.
* Significantly different from controls at $p \leq 0.05$

**Table 11b. Mean (±SD) sub-session motor activity (counts) in $F_1$ female pups in Subset 3 [a]**

| Block [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 14 | 1 | 19.2±16.6 | 23.7±22.3 | 19.2±20.3 | 13.3±11.8 |
| | 2 | 23.4±18.8 | 22.0±19.9 | 13.8±18.5 | 10.4±13.9 |
| | 3 | 26.0±16.6 | 23.7±26.5 | 21.3±23.4 | 15.0±14.2 |
| | 4 | 21.0±17.7 | 24.8±23.2 | 25.3±24.4 | 19.4±17.4 |
| | 5 | 21.8±18.3 | 24.7±25.7 | 17.0±22.3 | 23.0±22.4 |
| | 6 | 21.1±20.8 | 24.1±24.1 | 16.2±21.4 | 20.5±19.6 |
| | 7 | 21.8±21.6 | 19.5±19.1 | 13.8±18.4 | 18.6±16.2 |
| | 8 | 13.0±17.5 | 17.3±16.4 | 9.0±22.7 | 16.5±22.1 |
| | 9 | 16.8±19.0 | 23.3±23.8 | 11.8±19.4 | 14.0±17.2 |
| | 10 | 14.2±19.2 | 21.6±23.2 | 13.6±22.1 | 18.7±20.7 |
| | 11 | 12.0±15.6 | 19.6±25.4 | 20.9±26.2 | 20.8±19.1 |
| | 12 | 9.4±16.4 | 16.2±22.3 | 17.9±19.8 | 21.6±20.2 |

*(Table continues next page)*

| Block [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 18 | 1 | 30.8±23.3 | 36.5±24.5 | 23.9±26.3 | 41.2±29.1 |
| | 2 | 39.8±26.5 | 45.1±22.1 | 34.5±29.2 | 43.6±28.2 |
| | 3 | 49.4±30.5 | 44.3±20.4 | 45.8±29.0 | 45.6±28.5 |
| | 4 | 51.2±32.5 | 49.2±22.7 | 47.4±33.4 | 45.4±30.3 |
| | 5 | 45.9±30.3 | 42.3±24.7 | 44.8±30.5 | 45.0±28.0 |
| | 6 | 49.6±29.1 | 44.9±30.4 | 46.4±32.0 | 44.9±30.4 |
| | 7 | 41.8±29.5 | 39.3±26.4 | 38.9±31.4 | 39.6±31.7 |
| | 8 | 38.8±29.0 | 36.9±29.6 | 31.9±28.7 | 35.8±29.1 |
| | 9 | 31.8±33.4 | 40.2±26.5 | 43.3±35.3 | 36.4±30.9 |
| | 10 | 31.4±31.6 | 31.9±31.6 | 39.5±29.9 | 30.4±31.1 |
| | 11 | 32.4±32.6 | 28.8±33.6 | 43.8±32.9 | 33.1±31.9 |
| | 12 | 32.0±31.4 | 31.2±30.2 | 36.0±35.0 | 33.6±30.0 |
| PND 22 | 1 | 66.7±11.1 | 68.8±9.2 | 58.6±19.5 | 55.3±19.4 (↓17) |
| | 2 | 59.8±18.2 | 62.7±12.8 | 50.0±18.6 | 53.8±17.1 |
| | 3 | 55.4±22.6 | 56.4±20.3 | 47.8±19.7 | 49.8±23.3 |
| | 4 | 56.6±14.5 | 52.4±17.2 | 43.2±24.3 | 41.2±19.9* (↓27) |
| | 5 | 50.0±21.9 | 48.2±23.2 | 38.4±27.2 | 44.3±23.3 |
| | 6 | 51.1±18.0 | 46.2±26.4 | 41.4±24.3 | 45.2±19.2 |
| | 7 | 48.5±21.0 | 41.2±32.1 | 32.0±27.9 | 37.2±22.8 |
| | 8 | 46.6±21.0 | 40.4±28.4 | 35.4±25.3 | 43.4±26.9 |
| | 9 | 38.2±24.1 | 44.2±29.0 | 39.0±30.0 | 42.6±25.1 |
| | 10 | 35.0±25.7 | 38.5±27.8 | 27.5±27.9 | 39.1±28.6 |
| | 11 | 35.9±25.5 | 35.7±28.5 | 27.5±25.5 | 33.2±26.6 |
| | 12 | 35.8±33.0 | 29.2±29.0 | 16.1±21.4 | 33.6±27.5 |

*(Table continues next page)*

| Block [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 62 | 1 | 66.4±11.0 | 68.5±10.0 | 64.2±8.9 | 60.9±8.9 |
| | 2 | 68.4±8.9 | 70.4±11.0 | 70.2±8.0 | 64.8±9.8 |
| | 3 | 72.6±7.5 | 70.8±12.5 | 69.0±8.7 | 67.0±8.4 |
| | 4 | 71.0±9.0 | 69.0±10.0 | 68.9±8.9 | 63.2±10.4* (↓11) |
| | 5 | 71.3±14.1 | 68.7±9.2 | 67.8±12.6 | 64.2±19.0 |
| | 6 | 69.0±12.3 | 62.3±20.8 | 63.6±12.3 | 58.6±17.2 |
| | 7 | 63.0±12.6 | 60.1±21.7 | 62.0±15.0 | 58.2±20.8 |
| | 8 | 64.4±23.6 | 53.0±22.7 | 51.8±24.5 | 54.1±22.8 |
| | 9 | 55.4±27.0 | 40.5±30.2 | 54.4±23.2 | 48.7±25.5 |
| | 10 | 58.6±31.6 | 38.2±28.3 | 52.0±27.1 | 41.3±31.7 |
| | 11 | 46.7±30.6 | 34.3±30.4 | 44.2±28.3 | 32.6±26.2 |
| | 12 | 42.6±27.5 | 27.5±26.5 | 40.2±26.7 | 37.7±29.8 |

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically.
b Each block is a 5-minute period.
* Significantly different from controls at $p \leq 0.05$

**c. <u>Auditory startle reflex habituation</u>:** Auditory startle reflex magnitude data are summarized in Table 12. In the 1750 ppm females on PND 23, the average magnitude of response over 5 blocks and the mean response for each individual block were substantially decreased ($p \leq 0.01$) by 45-50% (average of 48%) as compared to controls. This finding was considered to be treatment-related. Decreases from control values (not significant) in the mean sub-session (block) values and the overall average magnitude of the auditory startle response were also observed on PND 23 in the 1750 ppm males (decreased by 17-34%; average of 29%) and in the 500 ppm females (decreased by 13-36%; average of 27%). These non-significant decreases observed in PND 23 pups were not judged to be related to treatment due to the following factors: 1) The sub-session (block) data for the 1750 ppm males and the 500 ppm females were not significantly different from control values, with the isolated exception of the second block of testing for the 500 ppm females ($p \leq 0.05$). 2) Examination of the overall average startle magnitude values across study groups did not reveal a dose-response relationship in the PND 23 male data; average response magnitude was <u>in</u>creased at 500 ppm. 3) The magnitude of the difference between the average response magnitude in treated pups and controls was similar for the 1750 ppm males (↓29%) and the 500 ppm females (↓27%). There were no apparent effects of treatment on auditory startle habituation on PND 63.

Historical control auditory startle habituation data from two developmental neurotoxicity studies previously conducted by the performing laboratory (study report pages 918 and 920) did not assist in the interpretation of the data for the DNT study with clothianidin. In this very limited historical database, reported average response magnitudes were 11.6 and 12.2 for PND 23 males and 12.5 and 11.6 for PND 23 females. Positive control data for auditory startle habituation were provided (see summary in Appendix A to this DER) but were not reported in sufficient detail to

aid in the interpretation of the study findings. Comparison of the PND 23 control data from the current study with the historical control data indicate that the female control values were higher than those seen historically.

**Table 12. Mean (±SD) auditory startle reflex magnitude (g) data from $F_1$ rats in Subset 3.** [a]

| Observation [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| Males | | | | | |
| PND 23 | Block 1 | 18.3±10.9 | 15.0±7.0 | 23.0±14.2 | 13.5±3.5 (↓26) |
| | Block 2 | 13.2±9.8 | 12.8±6.3 | 17.9±14.0 | 10.9±4.2 (↓17) |
| | Block 3 | 15.8±11.1 | 10.5±4.9 | 16.6±13.4 | 9.7±5.3 (↓39) |
| | Block 4 | 15.5±10.4 | 10.9±7.5 | 16.8±14.2 | 10.8±5.5 (↓30) |
| | Block 5 | 15.1±9.3 | 14.1±13.5 | 17.4±13.1 | 9.9±3.9 (↓34) |
| | Average | 15.6±8.5 | 12.6±6.3 | 18.3±12.8 | 11.0±3.6 (↓29) |
| PND 63 | Block 1 | 62.8±54.8 | 59.9±53.0 | 85.8±56.4 | 75.8±38.0 |
| | Block 2 | 39.2±49.8 | 34.8±40.4 | 55.9±53.9 | 46.2±25.5 |
| | Block 3 | 33.7±32.4 | 34.5±41.5 | 46.4±44.6 | 45.3±31.9 |
| | Block 4 | 30.5±24.9 | 28.7±26.5 | 39.3±26.4 | 34.2±25.0 |
| | Block 5 | 23.3±17.9 | 22.4±17.5 | 31.2±25.9 | 32.3±20.6 |
| | Average | 37.9±31.0 | 36.1±33.3 | 51.7±37.9 | 46.7±23.3 |
| Females | | | | | |
| PND 23 | Block 1 | 23.0±11.2 | 17.7±7.6 | 20.1±13.0 (↓13) | 12.7±5.8** (↓45) |
| | Block 2 | 20.7±12.9 | 16.1±8.1 | 13.3±7.5* (↓36) | 10.1±7.6** (↓50) |
| | Block 3 | 19.5±14.3 | 18.5±11.2 | 13.9±9.2 (↓29) | 10.0±6.8* (↓49) |
| | Block 4 | 21.0±14.2 | 17.4±12.4 | 14.1±9.8 (↓33) | 11.5±7.8* (↓45) |
| | Block 5 | 22.6±16.7 | 17.8±11.4 | 16.1±10.8 (↓29) | 11.2±6.2** (↓50) |
| | Average | 21.3±12.7 | 17.5±8.1 | 15.5±8.2 (↓27) | 11.1±5.7** (↓48) |
| PND 63 | Block 1 | 44.9±28.2 | 46.8±36.9 | 34.2±24.2 | 48.8±57.9 |
| | Block 2 | 30.7±22.1 | 28.3±24.2 | 22.4±14.3 | 33.6±29.3 |
| | Block 3 | 26.8±22.9 | 23.0±20.2 | 19.6±14.1 | 20.7±20.9 |
| | Block 4 | 22.6±15.5 | 16.6±18.8 | 14.2±11.0 | 23.0±25.5 |
| | Block 5 | 25.0±22.1 | 15.0±14.3 | 15.2±10.2 | 24.9±33.3 |
| | Average | 30.0±18.0 | 26.0±19.6 | 21.1±12.5 | 30.2±29.7 |

a Data obtained from MRID 45422804 Table F2, pages 466-467; n=18-20. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

b Block=10 consecutive trials

* Statistically different from control, $p \leq 0.05$

** Statistically different from control, $p \leq 0.01$

**d. Learning and memory testing:** Passive avoidance and water maze data are summarized in Tables 13 and 14, respectively). No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance or water maze tests. A nonsignificant 19% decrease in the latency time for trial 2 in the passive avoidance test for 1750 ppm males at PND 24 was not judged to be indicative of a treatment-related response.

**Table 13. Mean (±SD) passive avoidance performance data in $F_1$ rats [a]**

| Session/Parameter | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| Males | | | | | |
| Session 1 PND 23 | Trials to criterion | 3.6±0.8 | 3.8±0.8 | 4.0±0.9 | 3.7±0.9 |
| | Latency trial 1 (sec) | 10.0±8.8 | 13.5±13.5 | 9.5±9.8 | 13.8±16.6 |
| | Latency trial 2 (sec) | 50.4±16.3 | 40.9±22.2 | 37.0±21.0 | 40.6±20.5 (↓19) |
| | Failed to learn (n) | 0 | 0 | 0 | 0 |
| Session 2 PND 30 | Trials to criterion | 2.9±0.8 | 2.7±0.6 | 3.1±1.0 | 2.7±0.6 [b] |
| | Latency trial 1 (sec) | 33.2±24.2 | 29.0±23.2 | 30.6±25.4 | 35.4±22.2 [b] |
| Females | | | | | |
| Session 1 PND 24 | Trials to criterion | 3.6±0.5 | 4.0±1.2 | 3.6±0.7 | 3.6±1.0 |
| | Latency trial 1 (sec) | 12.8±9.5 | 12.3±14.8 | 15.5±14.3 | 14.8±13.3 |
| | Latency trial 2 (sec) | 40.8±20.2 | 35.2±23.6 | 39.9±19.9 | 44.2±19.6 |
| | Failed to learn (n) | 0 | 0 | 0 | 0 |
| Session 2 PND 31 | Trials to criterion | 2.8±0.8 | 3.4±2.1 | 3.2±1.2 | 2.8±0.7 [c] |
| | Latency trial 1 (sec) | 35.6±22.7 | 35.0±25.1 | 28.8±19.8 | 39.2±22.8 [c] |

a Data obtained from MRID 45422804 Table E1, page 421; n=18-20.
b Excludes values for rats found dead; n=19.
c Excludes values for rats found dead; n=18.

**Table 14. Mean (±SD) water maze performance data in $F_1$ rats [a]**

| Session/Parameter | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| Males | | | | | |
| Session 1 | Trials to criterion | 9.1±3.1 | 9.1±2.5 | 7.9±1.9 | 7.3±1.4 [b] |
| | Errors per trial | 0.43±0.18 | 0.43±0.25 | 0.35±0.19 | 0.31±0.15 [b] |
| | Latency trial 2 (sec) | 13.4±5.7 | 15.0±9.4 | 14.2±8.5 | 12.3±7.8 [b] |
| | Failed to learn (n) | 2 | 0 | 0 | 0 |
| Session 2 | Trials to criterion | 5.7±1.3 | 5.4±0.6 | 6.6±2.6 | 5.9±1.4 |
| | Errors per trial | 0.04±0.07 | 0.10±0.18 | 0.11±0.12 | 0.09±0.12 |
| | Latency trial 1 (sec) | 7.5±3.0 | 10.5±8.4 | 11.0±9.4 | 8.9±4.4 |
| Females | | | | | |
| Session 1 | Trials to criterion | 7.7±2.2 | 8.6±2.7 | 8.2±2.2 | 7.7±2.1 [c] |
| | Errors per trial | 0.46±0.32 | 0.41±0.38 | 0.44±0.22 | 0.44±0.24 [c] |
| | Latency trial 2 (sec) | 19.5±15.4 | 13.9±7.4 | 17.7±12.3 | 13.8±11.0 [c] |
| | Failed to learn (n) | 0 | 1 | 0 | 0 |
| Session 2 | Trials to criterion | 6.4±2.3 | 7.9±3.4 | 7.2±3.1 | 6.6±1.9 |
| | Errors per trial | 0.17±0.25 | 0.22±0.26 | 0.18±0.16 | 0.18±0.20 |
| | Latency trial 1 (sec) | 13.0±13.5 | 9.9±5.6 | 11.8±5.6 | 15.6±12.2 |

a Data obtained from MRID 45422804 Table E2, page 422; n=18-20.
b Excludes values for rats found dead; n=19.
c Excludes values for rats found dead; n=18.

## 5. Postmortem results

**a. Brain weights:** No treatment-related differences in brain weights or brain-to-body weight ratios were noted between treated and control groups for PND 12 pups or for offspring at termination (Table 15).

**Table 15. Mean (±SD) brain weights in $F_1$ rats [a]**

| Parameter | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Males | | | | |
| PND 12 (subset 1) | | | | |
| Terminal Body Weight (g) | 19.0±3.4 | 18.9±3.6 | 18.1±3.8 | 17.8±3.3 |
| Brain Weight (g) | 1.14±0.12 | 1.14±0.15 | 1.09±0.15 | 1.15±0.15 |
| Brain-to-body weight ratio (%) | 6.108±0.746 | 6.110±0.589 | 6.164±0.706 | 6.573±0.827 |
| Termination (subset 4) | | | | |
| Terminal Body Weight (g) | 429.1±45.0 | 427.1±31.7 | 425.7±38.0 | 405.1±30.5 |
| Brain Weight (g) | 2.229±0.131 | 2.291±0.085 | 2.200±0.118 | 2.170±0.119 |
| Brain-to-body weight ratio (%) | 0.522±0.047 | 0.539±0.037 | 0.519±0.035 | 0.537±0.028 |
| Females | | | | |
| PND 12 (subset 1) | | | | |
| Terminal Body Weight (g) | 17.2±3.1 | 17.8±4.1 | 17.0±2.1 | 16.8±2.6 |
| Brain Weight (g) | 1.08±0.13 | 1.08±0.17 | 1.06±0.08 | 1.11±0.12 |
| Brain-to-body weight ratio (%) | 6.407±0.728 | 6.233±0.714 | 6.251±0.454 | 6.710±0.460 |
| Termination (subset 4) | | | | |
| Terminal Body Weight (g) | 261.4±26.1 | 253.3±28.0 | 258.6±33.1 | 248.2±16.8 |
| Brain Weight (g) | 2.053±0.086 | 2.084±0.101 | 2.034±0.080 | 2.033±0.145 |
| Brain-to-body weight ratio (%) | 0.791±0.076 | 0.830±0.097 | 0.799±0.109 | 0.818±0.046 |

a Data obtained from MRID 45422804 Tables D3-D4 and G3-G4, pages 402-403 and 625-626; n=10 for subset 4 and 20 for subset 1.

**b. Macroscopic examination:** Gross necropsy findings at study termination revealed slight to marked dilation of the pelvis of the kidney in male rats at all treatment levels (3 males at 175 ppm, one male at 500 ppm, and one male at 1750 ppm). Additionally, a small, purple, and flaccid left testis and small, flaccid left epididymis was observed in one 1750 ppm male. No treatment-related gross pathological findings of the nervous system were noted in any treated group.

**c. Microscopic examination:**

**1) Urogenital system of male offspring:** The pelvic dilation of the kidneys observed at necropsy was confirmed histopathologically. Histopathological evaluation revealed diffuse, moderate

testicular degeneration and minimal focal mononuclear cell infiltration with mild, necrotic germ cell in the epididymis.

**2) Neurological system:** No treatment-related microscopic findings were noted at subjective histopathological evaluation of nervous system tissues. Morphometric evaluations (Table 16) revealed a number of significant differences from control at 1750 ppm. In the 1750 ppm females, differences ($p \leq 0.05$) were noted in the thickness of the hippocampal gyrus (↑9%), cerebellum height (↑10%), and the external germinal layer of the cerebellum (↓11%) on PND 12. Additionally, on PND 83-87, decreases ($p \leq 0.05$) in the thickness of the caudate putamen (↓6%) and hippocampal gyrus (↓5%) were noted in the 1750 ppm females. Although the measurements of the hippocampus demonstrate an increase in weanlings, and a decrease in adults, their relationship to treatment was nevertheless considered to be biologically plausible. This could be explained if the effects noted at termination are not representative of a persistence of effects, but are rather the latent consequences of early exposure.

Due to the presence of apparently treatment-related findings at 1750 ppm, morphometric evaluations were conducted in PND 12 and adult 500 ppm females; no significant differences from control were observed. A dose-response relationship was noted in the responses observed in the female cerebellum height at PND 12 and in the hippocampal gyrus at PND 12 and termination; for these findings, a similar trend was noted in males, although no significance was identified. The PND 12 male hippocampal gyrus thickness and cerebellum height were each increased 4% from control, and the termination hippocampal gyrus thickness was decreased 5% from control. In summary, the following findings were judged to be related to treatment at 1750 ppm: the increased thickness of the hippocampal gyrus (both sexes), the increased thickness of the cerebellum height (both sexes), and the decreased thickness of the external germinal layer at PND 12 (females), and the decreased thickness of hippocampal gyrus (both sexes) and caudate putamen (females) at termination.

**Table 16. Mean (±SD) morphometric data for $F_1$ rats [a]**

| Parameter | Dose (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 | 0 | 150 | 500 | 1750 |
| | Males | | | | Females | | | |
| PND 12 (subset 1) | | | | | | | | |
| Cerebrum (mm) | 12.14±0.59 | NA | NA | 12.35±0.50 | 11.97±0.56 | NA | 11.94±0.46 | 12.14±0.57 |
| Cerebellum (mm) | 5.40±0.47 | NA | NA | 5.55±0.50 | 5.28±0.60 | NA | 5.32±0.43 | 5.49±0.57 |
| Frontal Cortex (µm) | 1356.0±97.5 | NA | NA | 1413.6±114.0 | 1356.0±132.1 | NA | 1377.0±83.0 | 1416.0±141.3 |
| Parietal Cortex (µm) | 1408.8±68.9 | NA | NA | 1461.6±102.7 | 1423.2±70.7 | NA | 1431.0±56.7 | 1488.0±86.9 |
| Caudate Putamen (µm) | 2548±185.8 | NA | NA | 2568.0±118.1 | 2529.6±128.1 | NA | 2442.0±102.2 | 2572.8±158.7 |
| Corpus Callosum (µm) | 282.1±32.6 | NA | NA | 293.9±31.0 | 261.1±37.3 | NA | 288.0±29.4 | 273.5±56.5 |
| Hippocampal Gyrus (µm) | 948.0±42.7 | NA | NA | 981.6±88.0 (↑4) | 919.2±67.9 | NA | 942.0±69.6 | 1003.2±81.4* (↑9) |
| Cerebellum Height (µm) | 3004.8±340.3 | NA | NA | 3129.6±309.3 (↑4) | 2856.0±277.4 | NA | 2946.0±194.8 | 3148.8±259.2* (↑10) |
| Ext. Germinal Layer (µm) | 34.3±4.2 | NA | NA | 33.9±3.3 | 37.2±3.5 | NA | 38.1±4.0 | 33.2±4.9* (↓11) |
| Termination (subset 4) | | | | | | | | |
| Cerebrum (mm) | 15.83±0.27 | NA | NA | 15.66±0.30 | 15.59±0.28 | NA | 15.48±0.28 | 15.53±0.56 |
| Cerebellum (mm) | 7.09±0.36 | NA | NA | 7.18±0.39 | 7.25±0.24 | NA | 7.17±0.37 | 7.15±0.28 |
| Frontal Cortex (µm) | 1848.4±123.9 | NA | NA | 1893.6±165.7 | 1711.2±118.1 | NA | 1710.0±78.7 | 1730.4±107.6 |
| Parietal Cortex (µm) | 1956.0±105.7 | NA | NA | 1992.0±160.8 | 1800.0±35.8 | NA | 1758.0±63.6 | 1795.2±71.4 |
| Caudate Putamen (µm) | 3542.4±218.0 | NA | NA | 3700.8±221.1 | 3379.2±206.4 | NA | 3480±174.4 | 3192.0±155.5* (↓6) |
| Corpus Callosum (µm) | 281.4±37.8 | NA | NA | 272.6±30.1 | 266.9±43.7 | NA | 266.4±38.7 | 253.5±26.5 |
| Hippocampal Gyrus (µm) | 1819.2±68.6 | NA | NA | 1735.2±112.0 (↓5) | 1562.4±69.2 | NA | 1506±73.2 | 1483.2±94.5* (↓5) |
| Cerebellum Height (µm) | 5419.2±444.8 | NA | NA | 5424.0±569.3 | 4915.2±267.0 | NA | 4926±114.7 | 4771.2±255.2 (↓3) |

a Data obtained from MRID 45422804, pages 765-770 and 816-821; n=10. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

* Statistically different from control, $p \leq 0.05$

NA Not applicable

## III. DISCUSSION and CONCLUSIONS

**A. INVESTIGATORS' CONCLUSIONS:** The investigators concluded that maternal toxicity at 1750 ppm was characterized by decreased body weight and food consumption. Developmental and/or neonatal toxicity at ≥500 ppm was characterized by decreases in body weight gains during pre-weaning, motor activity on PND 22, and acoustic startle response in females on PND 23. The maternal NOAEL was 500 ppm. The offspring NOAEL was 150 ppm.

**B. REVIEWER'S COMMENTS:** In this study, maternal toxicity was observed as decreased body weight, body weight gain, and food consumption during gestation and lactation at 1750 ppm. Maternal body weights were consistently decreased ($p \leq 0.05$ or NS) throughout gestation and lactation (↓2-8%). Body weight gains were decreased ($p \leq 0.05$) during GDs 0-3 (↓63%) and

LDs 4-7 (↓67%). These decreases corresponded with the significant reductions noted in absolute (↓7-23%) and relative (↓6-22%) food consumption during the gestation and lactation periods.

Overall effects on maternal body weight gain and food consumption at 1750 ppm were minimal in nature. Decreased body weight and body weight gain were correlated with decreased food consumption, suggesting that there was little difference in food efficiency between control and treated rats (not calculated). At 1750 ppm, decreased body weight gain and food consumption from GD 0-3 is suggestive of a palatability problem. Body weight was essentially recovered by the end of gestation. Decreased body weight gain during lactations days 4-7 was similar for the low (150 ppm) and high (1750 ppm) dose groups (i.e., there was no dose-response), and body weight gain was substantially increased at 500 and 1750 ppm during lactation days 14-22 (i.e., when pups would have also been beginning to consume treated feed). Overall gestation (GD 0-20) and lactation (LD 1-22) body weight gains were not statistically different from control for any treated group.

**The maternal LOAEL is 1750 ppm (142 mg/kg/day) based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 500 ppm (42.9 mg/kg/day).**

During the pre-weaning period, significantly decreased body weight was noted on PND 14-22 in the females pups at 500 ppm (↓6-7%) and in both sexes at 1750 ppm (↓6-16%). Body weight gains were decreased ($p \leq 0.05$) in the female pups at 500 ppm (↓8-24%) and in both sexes at 1750 ppm (↓11-48%). Overall pre-weaning (PND 5-22) body weight gains were decreased ($p \leq 0.05$) by 7% in the 500 ppm females and by 18% at 1750 ppm in both sexes. Immediately after weaning, decreased ($p \leq 0.05$) body weights were noted at 1750 ppm in both sexes (↓4-15%). Also during this time period, two male and three female rats at 1750 ppm were found dead on PNDs 25-27; this finding was considered related to a failure to thrive post-weaning, based on the patterns of body weight gains and losses. However, within 3 weeks post-weaning (i.e., following the cessation of treatment) the treatment-related body weight deficits in 1750 ppm pups had essentially been reversed.

There were no effects of treatment on other developmental landmarks examined, including pinna unfolding, acoustic startle response, eye opening, pupil constriction, balanopreputial separation or vaginal patency. A slight retardation in surface righting reflex was observed on PND 3 in all treated groups compared to controls; however, following a careful examination of the data and methodologies used, it was concluded that this finding could not be unequivocally attributed to treatment.

In a modified functional observational battery, no clinical findings consistent with "abnormal autonomic functions" in offspring were observed.

There were no apparent effects of treatment in motor activity data measured on PND 14, 18, or 62. On PND 22, mean total motor activity (number of movements) was decreased (NS) in the 1750 ppm males (↓24%) and in the females at 500 ppm (↓21%) and 1750 ppm (↓10%). The mean time spent in movement on PND 22 was also decreased (NS) in the 1750 ppm males (↓27%) and in the females at 500 ppm (↓24%) and 1750 ppm (↓19%). The study author

considered all of these effects to be evidence of treatment-related toxicity. Overall, interpretation of the motor activity data is complicated by the variability in the data. Nevertheless, the decreases in motor activity counts and the time spent in movement at PND 22 for 1750 ppm male offspring are of substantial magnitude and supported by sub-session data; therefore, they were judged to be related to treatment. Agency reviewers considered the decreases in motor activity in 1750 ppm female weanlings to be minimal in nature and not likely to be treatment-related. Additionally, it was noted that effects noted for 500 ppm female offspring at PND 22, while nearly of the same magnitude as those observed in PND 22 males at 1750 ppm, demonstrated a lack of dose-response and an absence of significant corollary alterations in the sub-session data. Therefore, the apparent decreases in motor activity in PND 22 females at 500 ppm were also not considered to be treatment-related by Agency reviewers.

In general, habituation was not observed in the motor activity data on PND 14 or 18, but some habituation was evident in both male and female offspring on PND 22 and PND 62. There did not appear to be any treatment-related differences in habituation between control and treated rats. On all of the testing days, individual motor activity data demonstrated some animals with little or no habituation.

In the 1750 ppm females on PND 23, the average magnitude of the acoustic startle response over 5 blocks and the mean response for each individual block were substantially decreased ($p \leq 0.01$) by 45-50% (average of 48%) as compared to controls. This finding was considered to be treatment-related. Decreases from control values (not significant) in the mean sub-session (block) values and the overall average magnitude of the auditory startle response were observed on PND 23 in the 1750 ppm males (decreased by 17-34%; average of 29%) and in the 500 ppm females (decreased by 13-36%; average of 27%). These non-significant decreases observed in PND 23 pups were not judged to be related to treatment since: 1) the sub-session (block) data for the 1750 ppm males and the 500 ppm females were not significantly different from control values, with the isolated exception of the second block of testing for the 500 ppm females ($p \leq 0.05$). 2) examination of the overall average startle magnitude values across study groups did not reveal a dose-response relationship in the PND 23 male data; average response magnitude was increased at 500 ppm, and 3) the magnitude of the difference between the average response magnitude in treated pups and controls was similar for the 1750 ppm males (↓29%) and the 500 ppm females (↓27%). There were no apparent effects of treatment on auditory startle habituation on PND 63.

No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance or water maze tests.

There were no treatment-related differences in brain weights or brain-to-body weight ratios between control and treated groups for offspring at PND 12 or at termination (PND 83-87). No treatment-related macroscopic findings were noted at necropsy and no treatment-related microscopic findings were noted at subjective histopathological evaluation of nervous system tissues. Morphometric evaluations revealed a number of significant differences from control at 1750 ppm. In the 1750 ppm females, differences ($p \leq 0.05$) were noted in the thickness of the hippocampal gyrus (↑9%), cerebellum height (↑10%), and the external germinal layer of the cerebellum (↓11%) on PND 12. Additionally, on PND 83-87, decreases ($p \leq 0.05$) in the

thickness of the caudate putamen (↓6%) and hippocampal gyrus (↓5%) were noted in the 1750 ppm females. Morphometric evaluations of PND 12 and adult 500 ppm females revealed no significant differences from control. A dose-response relationship was noted in the responses observed in the female cerebellum height at PND 12 and in the hippocampal gyrus at PND 12 and termination; for these findings, a similar trend was noted in males, although no significance was identified. The PND 12 male hippocampal gyrus thickness and cerebellum height were each increased 4% from control, and the termination hippocampal gyrus thickness was decreased 5% from control. In summary, based upon the magnitude and/or statistical significance of the response and/or on apparent dose-response relationships, the following findings were judged to be related to treatment at 1750 ppm: the increased thickness of the hippocampal gyrus (both sexes), the increased thickness of the cerebellum height (both sexes), and the decreased thickness of the external germinal layer at PND 12, and the decreased thickness of hippocampal gyrus (both sexes) and caudate putamen (females) at termination.

**The offspring systemic LOAEL is 500 ppm (42.9 mg/kg/day), based on decreased body weights and body weight gains of female pups during PND 14-21. The offspring systemic NOAEL is 150 ppm (12.9 mg/kg/day).**

**The offspring neurotoxicity LOAEL is 1750 ppm (142 mg/kg/day), based on decreased motor activity (number and duration of movements) in PND 22 male pups, decreased magnitude of the auditory startle response in PND 23 females, increases in the thickness of the hippocampal gyrus and cerebellum height and decreases in the external germinal layer in the brains of PND 12 pups; and decreases in the thickness of the caudate putamen and hippocampal gyrus in adult offspring at termination (PND 83-87). The offspring neurotoxicity NOAEL is 500 ppm (42.9 mg/kg/day).**

This study is classified as **acceptable/non-guideline** and does not satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats. Classification may be upgradable to guideline upon submission of procedural information for functional observation assessments.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted:
1) The method used for detection of functional changes was not adequately described in the text of the report. The procedures used were not described, including whether the same technicians were used throughout testing, where the testing was done (including whether the animals were removed from the cage), when testing was done with respect to time of test substance administration, what the environmental conditions were (e.g., noise level, etc.), whether scoring criteria were used for the measured parameters, the duration of the observation period for open field observations. There was no mention of evaluation of pupillary function such as constriction of the pupil in response to light, or a measure of pupil size.
2) According to the histopathology report, gross morphometric measurements (A/P cerebrum and cerebellum) were performed on all brains evaluated histopathologically, however data were reported only for high dose and control animals, and any mid-dose females that were processed for further analysis. In addition, morphometric measurements were made bilaterally for a number of areas of the brain in both PND 12 and adult offspring (see report pages 756 and 811),

but only the mean values were reported. Values for morphometric measurements evaluated, but not included in the current report, should be submitted.
3) Latency was not reported for auditory startle data.

Appendix A – Positive Control Data

The following positive control data were provided:

Parker, R. (1999) Neurotoxicity evaluation of positive control substances in Crl:CD®(SD)IGS BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-075, August 6, 1999. Unpublished. This study used a functional observational battery (FOB) to evaluate the positive control substances acrylamide, trimethyltin, MK-801, carbaryl, and DDT and was not acceptable for use with the current study because the current study did not use a formal FOB. (MRID 45422804, pp. 959-1137)

Foss, J. (1992) Neurotoxicity evaluation of DDT in Crl:CD®(SD)IGS BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-015. Unpublished. This study used an FOB to evaluate the positive control substance DDT and was not acceptable for use with the current study because the current study did not use a formal FOB. (MRID 45422804, pp. 1138-1147)

Lochry, E., J. Foss, and M. Christian. (1990) Validation of a functional observational battery and motor activity measure using positive control substances. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 11th annual meeting of the American College of Toxicology; Orlando, Florida; October, 1990. This study used an FOB to evaluate the positive control substances DDT, physostigmine monosalicylate, or acrylamide and used a motor activity assessment to evaluate the positive control substances chlorpromazine or amphetamine. It was not acceptable for use with the current study because the current study did not use a formal FOB, and because the motor activity sessions in the positive control study were 2 hours in duration and comprised of 24 5-minute blocks, while the current study used 1-hour sessions comprised of twelve 5-minute blocks. There was also insufficient information provided to determine whether the same equipment was used as was used in the current study. (MRID 45422804, pp. 1148-1181)

Foss, J. (1991) Neurotoxicity evaluation of positive control substances in Crl:CD® VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-014. Unpublished. This study used an FOB and motor activity assessment to evaluate the positive control substances acrylamide, IDPN, carbaryl, DDT, and triadimefon. It was not acceptable for use with the current study because the current study did not use a formal FOB, and because the motor activity sessions in the positive control study were 1.5 hours in duration/comprised of 18 5-minute blocks, while the current study used 1-hour sessions comprised of twelve 5-minute blocks. There was also insufficient information provided to determine whether the same equipment was used as was used in the current study. (MRID 45422804, pp. 1182-1249)

Foss, J. and E. Lochry (1991) The assessment of motor activity in neonatal and adult rodents using passive infrared sensors. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 12th annual meeting of the

American College of Toxicology; Savannah, Georgia; October, 1991. This study used passive infrared sensors to monitor motor activity of untreated adult rats, untreated adult mice, and neonatal rats on postnatal days 13, 17, 21, and 58-59. The positive control substances d-Amphetamine and chlorpromazine were evaluated in rats at approximately postnatal day 60, and the positive control substances acrylamide, IDPN, carbaryl, DDT, and triadimefon were evaluated in adult rats. Test sessions with positive control substances were 90-115 minutes in duration and comprised of 5-minute blocks, while the current stuy used 1-hour sessions comprised of twelve 5-minute blocks. (MRID 45422804, pp. 1250-1257)

Neurotoxicity evaluation of positive control substances in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-058. Unpublished. This study used motor activity assessment, auditory startle habituation, and neurohistological examination to evaluate the positive control substances acrylamide, trimethyltin chloride, or MK-801. Motor activity assessment was conducted using similar equipment to that used in the current study; however, sessions were 1.5 hours in duration and comprised of 5 minute blocks, while the current study used 1-hour sessions comprised of 10-minute blocks. Auditory startle habituation testing was conducted using similar equipment and methods as those used in the current study. Similar processing and staining methods were used, and the positive control study evaluated the same brain sections for neuropathology as those evaluated in the $F_1$ adults in the current study. (MRID 45422804, pp. 1258-1296)

Lochry, E. and E. Riley (1980) Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. Neurobehavioral Toxicology, Vol. 2, pp. 107-115. This study used different equipment than that used in the current study to assess passive avoidance and learning acquisition and retention and is not acceptable for use as positive control data. (MRID 45422804, pp. 1297-1306)

Lochry, E., J. Foss, and M. Christian (1990) Learning and retention paradigms in developmental neurotoxicity test batteries: passive avoidance and water maze. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 18th European Teratology Society Conference; Edinburgh, Scotland; September 1990. This was a collection of historical control data from passive avoidance and water maze testing conducted in 1988-1989. No further details were provided. (MRID 45422804, pp. 1307-1312)

Foss, J., E. Lochry, and A. Hoberman (1990) Automated monitoring systems for motor activity and auditory startle applicable for both developmental and adult neurotoxicity studies. Poster presented at the 8th International Neurotoxicity Conference; Little Rock, Arkansas; October, 1990. Motor activity was assessed on postnatal days 13, 17, 21, and 60, using similar equipment to that used in the current study; however, the test session was 1.5 hours long and comprised of 18 5-minute blocks, while the current study used 1 hour test sessions comprised of twelve 5-minute blocks. Auditory startle habituation was assessed on postnatal days 22 and 60, using similar equipment and methods to those used in the current study. (MRID 45422804, pp. 1313-1327)

Foss, J. and E. Riley (1989) Elicitation and modification of the acoustic startle reflex in animals prenatally exposed to cocaine, Neurotoxicology and Teratology 13:541-546. This study was conducted using different equipment than that used in the current study and is not acceptable for use as positive control data for the current study. (MRID 45422804, pp. 1328-1334)

E. Lochry, A. Hoberman, and M. Christian (1985) Detection of prenatal effects on learning as a function of differential criteria, Neurotoxicology and Teratology 7:697-701. This study was conducted using different equipment than that used in the current study and is not acceptable for use as positive control data for the current study. (MRID 45422804, pp. 1335-1340)

Garman, R.H. (1998) Neuropathology Validation Report, Consultants in Veterinary Pathology, Inc., P.O. Box 68, Murrysville, PA 15668, dated 8-21-96. Unpublished. This consisted of a brief description of the consulting neuropathologist's credentials, experience, and publications, and also included the neuropathology methods and results from Argus Research Laboratories, laboratory project number 012-058 [mentioned above]. (MRID 45422804, pp. 1341-1390)

Garman, R.H. (1998) Morphometric measurement validation study comparing day 10 and day 12 pups Report, Consultants in Veterinary Pathology, Inc., P.O. Box 68, Murrysville, PA 15668. Unpublished. This study compared 9 different morphometric measurements between 10 and 12 day old pups. The brains were measured grossly and sectioned similarly to those of the PND 12 pups used in the current study. It was concluded that increases in the thickness of the frontal cortex, height of the cerebellar cortex, and cross-sectional width of the caudate-putamen correlated best with brain maturation between PND 10 and 12. Only the previous two of these three measurements were used in the current study, which also included measurement of the dentate gyrus of the hippocampus. (MRID 45422804, pp. 1391-1398)

Foss, J., A. Hoberman, and M. Christian (1992) Developmental neurotoxicity evaluation of lead nitrate in in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the Annual Meeting of the Society of Toxicology; Seattle, Washington; February 1992. Motor activity assessment was conducted using similar equipment to that used in the current study; however, the test session was comprised of 5-minute blocks, while the current study used 10-minute blocks. The equipment and methods used for auditory startle habituation, passive avoidance, and water maze testing were similar to those used in the current study, however no effects of treatment were detected. (MRID 45422804, pp. 1399-1412)

# DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

| PC code | MRID # | Study type | Species | Duration | Route | Dosing method | Dose range ppm | Doses tested mg/kg/day | NOAEL mg/kg/day | LOAEL mg/kg/day | Endpoint(s) | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 044309 | 45422804 | dev neurotox | rats | GD 0-LD 22 | oral | dietary | 0, 150, 500, 1750 | 0, 12.9, 42.9, 142 | 42.9 | 142 | decr BW, BWG, FC | Maternal |
| 044309 | 45422804 | dev neurotox | rats | GD 0-LD 22 | oral | dietary | 0, 150, 500, 1750 | 0, 12.9, 42.9, 142 | 12.9 | 42.9 | decr BW, BWG in female pups (PND 14-21) | Offspring systemic |
| 044309 | 45422804 | dev neurotox | rats | GD 0-LD 22 | oral | dietary | 0, 150, 500, 1750 | 0, 12.9, 42.9, 142 | 42.9 | 142 | decr motor activity in PND 22 males; decr auditory startle amplitude in PND 23 females; alterations in brain morphometry in PND 12 and 83-87 offspring | Offspring neurotox |